ADRENAL CORTEX

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Edited by

ELAINE P RALLI M D
ASSOCIATE PROFESSOR OF MEDICINE
COLLEGE OF MEDICINE
NEW YORK UNIVERSITY
NEW YORK N Y

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Transactions of the Third Conference on Adrenal Cortex

1 Table X on page 112 for orally read intramuscularly

2 In Figure 33 (page 138) and Table XVIII (page 142) and throughout the presentation of Oscar M Hechter The Biogenesis of Adrenal Cortical Steroids (pages 115 through 155) for pregneninolone read pregnenolone

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PARTICIPANTS

Fourth Conference on Adrenal Cortex

MEMBERS

C N HUGH LONG *Chairman*
Department of Physiology Yale University School of Medicine
New Haven Conn

ELAINE P RALLI *Secretary*
Department of Medicine New York University College of Medicine
New York N Y

EDWIN B ASTWOOD
Department of Medicine Tufts College Medical School
Boston Mass

WALTER BAUER*
Medical Division Massachusetts General Hospital
Boston Mass

KONRAD BLOCH*
Department of Biochemistry and Institute of Radiobiology and Biophysics
University of Chicago The School of Medicine
Chicago Ill

JEROME W CONN
Department of Internal Medicine University of Michigan Medical School
Ann Arbor Mich

ERNST GELLHORN
Department of Neurophysiology University of Minnesota Medical School
Minneapolis Minn

DWIGHT J INGLE
Research Laboratories The Upjohn Company
Kalamazoo Mich

EDWARD C KENDALL
The James Forrestal Research Center Princeton University
Princeton N J

CHOH HAO LI
Department of Biochemistry University of California
Berkeley Calif

ROBERT F LOEB*
Department of Practice of Medicine
Columbia University College of Physicians and Surgeons
New York N Y

GREGORY PINCUS
Worcester Foundation for Experimental Biology
Shrewsbury Mass

ROBERT F PITTS
Department of Physiology Cornell University Medical College
New York N Y

*Absent

GEORGE SAYERS
Department of Physiology Western Reserve University School of Medicine
Cleveland Ohio

HANS SELYE
Institute of Experimental Medicine and Surgery University of Montreal
Montreal Quebec Canada

GEORGE W THORN
Department of Medicine Peter Bent Brigham Hospital
Boston Mass

ABRAHAM WHITE
Department of Biochemistry
Columbia University College of Physicians and Surgeons
New York N Y

GUESTS

I E BUSH
National Institute for Medical Research
London England

OTTO LOEWI
Department of Pharmacology New York University College of Medicine
New York N Y

F D W LUKENS
George S Cox Medical Research Institute University of Pennsylvania
Philadelphia Pa

MARTHE VOGT
Pharmacological Laboratory University of Edinburgh
Edinburgh Scotland

The Josiah Macy Jr Foundation

FRANK FREMONT SMITH *Medical Director*

JANET FREED *Assistant for the Conference Program*

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JOSIAH MACY, JR FOUNDATION CONFERENCE PROGRAM

As an introduction to these Transactions of the Fourth Conference on Adrenal Cortex I should like to outline what it is that the Foundation hopes to accomplish by its Conference Program We are interested first of all in furthering knowledge about the adrenal cortex and to this end the participants were brought together to exchange ideas experiences data and methods In addition to this particular goal however there is a further and perhaps more fundamental aim which is shared by all our conference groups This is the promotion of meaningful communication between scientific disciplines

The problem of communication between disciplines we feel to be a very real and urgent one the most effective advancement of the whole of science being to a large extent dependent upon it Because of the accelerating rate at which new knowledge is accumulating and because discoveries in one field so often result from information gained in quite another channels must be established for the most effective dissemination and exchange of this knowledge

The increasing realization that nature itself recognizes no boundaries makes it evident that the continued isolation of the several branches of science is a serious obstacle to scientific progress Particularly is it true in medicine that the limited view through the lens of one discipline is no longer enough For example today medicine must be well versed in nuclear physics because of the tracer techniques and the injury which can result from radiation At the other extreme medicine is certainly *a social science and through mental health must be concerned with* economic and social questions The answer then is not further fragmentation into increasingly isolated specialties disciplines and departments but the integration of science and scientific knowledge for the enrichment of all branches This integration we feel can be encouraged by providing opportunities for a multiprofessional approach to given topics

Although the fertility of the multidiscipline approach is recognized adequate provision is not made for it by our universities scientific societies and journals And perhaps the presence of other hindering factors must be admitted Partly semantic in nature they may also to

some degree be psychological Admittedly it is oftentimes difficult to accept data derived from methods with which one is unfamiliar By making free and informal discussion the central core of our meetings we hope to achieve an atmosphere which minimizes as much as possible these semantic and emotional barriers

Thus our conferences are in contrast to the usual scientific gatherings Presentations are not designed to present neat solutions to tidy problems but rather to elicit provocative discussion of the difficulties which are being encountered in research and practice We ask that the presentations be relatively brief and emphasis is placed upon discussion as the heart of the meeting Our hope is that the participants will come prepared *not to defend a single point of view but with open minds* to take full advantage of the meeting as an opportunity to speak with representatives of other disciplines in much the same way as they talk with their colleagues in their own laboratories

During 1953 under the Conference Program conferences will be held on the following topics administrative medicine adrenal cortex aging connective tissues consciousness cybernetics infancy and childhood liver injury metabolic interrelations nerve impulse renal function and shock and circulatory homeostasis

When a new conference is organized the Chairman in consultation with the Foundation selects fifteen scientists to be the nucleus of the group which will hold annual meetings for a period of five years Every effort is made to include representatives from all pertinent disciplines From time to time however new members are added by the group to fill gaps in viewpoint or techniques A small number of guests is invited to attend each meeting but for the purposes of promoting full participation by all members and guests attendance at any meeting is limited to twenty five During a conference s prescribed lifetime we cannot possibly include more than a small fraction of the key investigators in the field and one of the difficulties in forming a group such as this one on the adrenal cortex is that it is necessary to exclude so many investigators we should like to include

The transactions of these meetings are recorded and published This is done because the Foundation wishes to make current thinking in a field available to all those working in it and to those in other fields who are concerned with science for example government officials administrators etc Logic is a vital aspect of science but equally essential is the intuitive or creative aspect Research is as creative as the painting of a portrait or the composing of a symphony Although logic is of course necessary in order to rearrange to test and to validate research thrives on creativity which has its source in unconscious nonrational processes Unfortunately however in the research reports which are

presented to the world in scientific journals this integral part of scientific endeavor is shriveled by the cold white light of logic By preserving the informality of our conferences in the published transactions we hope to portray more accurately what goes on in the minds of scientists and to give a truer picture of the role which creativity plays in scientific research

FRANK FREMONT SMITH M D
Medical Director

PERMISSIVE ACTION OF ADRENAL CORTICAL HORMONES

INFORMAL DISCUSSION

Long Departing from our usual procedure the discussion at this opening session will not be based on a presentation made by one of the participants but will have as its impetus unfinished business left over from previous meetings of the group I have re read the reports of the former conferences and have jotted down in no particular order topics that may be interesting to explore further For example in the discussion of Dr Pitts paper last year Dr Ingle spoke of what has been termed the permissive action of the adrenal cortical hormones As I understand it this term means that in the total absence of any adrenal hormones in the body there are a great many actions normally carried out by the cells metabolic processes of one kind or another that either proceed at very slow rate or else exhibit marked deficiencies The administration of minimal amounts of adrenal hormones—perhaps we can call them basal amounts—enable the cells to carry out a large number of reactions of various kinds which they cannot do in the absence of the hormone

The point that interests me is the comparison of this so called permissive action with what happens at the other extreme when an excess of the hormone is given I wonder in how many instances we are working with definite types of phenomena whether in most of these instances a reaction is not merely on a curve beginning with a certain minimal requirement and then as the amount is increased proceeding to a greater and greater degree until an area is reached which we recognize as the excessive action of the hormone Perhaps I might quote from some of Dr Ingle s work As he has demonstrated there are instances in which an adrenalectomized animal that has been partially or totally depancreatized will show when given small quantities of adrenal hormone and then placed in a particular circumstance an increase in glycosuria which obviously could not have occurred as a result of any increase in these hormones And yet as we all know and as Dr Ingle has also shown when an animal is given an excess of any of these hormones the net result is also an increase in glycosuria It may be worth while to discuss this interesting phenomenon further since it obviously might not only happen with an induced glycosuria but also in many other circumstances with which we have come to believe the adrenal hormones are concerned Dr Ingle what are your present views on the problem?

great many deficiencies in which the action of a hormone is not apparent One of the best examples is the effects of the growth hormone As far as I know if an animal is put in any situation in which the rate of catabolism is increased nitrogen loss occurs whether it is due to amino acid deficiency or any other deficiency Under these circumstances there probably will not be growth stimulation from administered growth hormone Yet if vitamin B deficiency is involved adding the minimal amount of the vitamin will again allow the hormone to display its normal effects The point you have raised with the adrenal cortical steroids seems to fall in this general class that is in the face of many types of deficiency the normal effects of the hormone are not exhibited I was particularly interested that giving the estrogen plus the adrenal cortical steroid is the same as giving a large excess of the adrenal cortical steroid

Ingle When cortisone and estrogen are combined a marked glycosuria and hyperglycemia is induced in normal animals under conditions in which neither estrogen nor cortisone alone would cause glycosuria (4) This may support the hypothesis that there is a continuous curve of sensitivity to cortical hormones

We have studied related problems during the past year Any sort of severe stress increases the secretory activity of the adrenal cortices and we have been interested in the metabolic consequences of adrenal cortical activation during stress Offhand one would expect that any stress which causes adrenal cortical activation should cause exacerbation of diabetes but we have found only one stressful agent which is diabetogenic Exercise causes the glycosuria of the diabetic animal to decrease Exposure of the diabetic rat to cold is followed by a very gradual fall in the level of glycosuria and when exposure to cold is stopped the glycosuria gradually rises to its prestress level The slowness of these changes is surprising Most toxic agents either have no significant effect upon glycosuria or they cause a slight decrease

The one toxic agent which causes exacerbation of diabetes in the partially depancreatized force fed rat is ethylenediamine (5) For these studies a very mildly diabetic rat maintained on 4 to 5 ml of adrenal cortical extract per day following adrenalectomy is used Ethylenediamine *in the adrenalectomized depancreatized rat treated with a uniform* intake of adrenal cortical extract did not result we found in an exacerbation of the diabetes Several indirect lines of evidence would support the conclusion that the diabetogenic effect of ethylenediamine is mediated by the adrenal cortex but ethylenediamine will exacerbate diabetes in the absence of the adrenal glands

We have a hypothesis as to what happens It is known that diabetes becomes less severe during states of adrenal cortical insufficiency It is

Ingle The permissive action of the adrenal cortical hormones can be illustrated by the relationship of the diabetogenic effect of estrogen to the adrenal cortex in the rat (1) Since certain of the adrenal cortical hormones are diabetogenic and since large doses of estrogen are among the noxious stimuli which cause the adrenal cortices to en large it seemed probable that the exacerbation of experimental diabetes by estrogen was due to stimulation of the anterior pituitary adrenal cortex axis which produced a state of hypercorticalism This hypothesis was supported by the observation that estrogen had little or no effect upon glycosuria in the adrenally insufficient, partially depancreatized rat However when such adrenalectomized rats were treated with a *moderate uniform intake of adrenal cortical extract during the entire* experiment the addition of estrogen caused a full blown exacerbation of the diabetes as evidenced by hyperglycemia and glycosuria

I formerly thought of the permissive action of the adrenal cortical hormones as an all or none effect More recently it has seemed prob able as Dr Long has suggested that there is a dose relationship to responsiveness at least for certain metabolic processes Most of our studies are concerned with the role of the adrenal cortex in certain metabolic adjustments to stress The hormones of the adrenal cortex play a permissive rather than a regulatory role in some and perhaps many adjustments to stress (2) Since the need for these hormones is greatly increased by severe stress it might be expected that increased amounts of hormones would be required to support a state of eucorti calism and consequently permissive action during severe as compared to a mild stress We have data which seem to require such assumptions

How the diabetogenic effect of adrenal cortical hormone overdosage relates to the permissive action of these same hormones in supporting the extra adrenal diabetogenic action of other agents remains a puzzling problem The depancreatized animal becomes diabetic because it lacks insulin not because of altered adrenal cortical function yet when the adrenal glands are removed from the diabetic animal there is striking amelioration of the diabetes (3) The cortical hormones must be pres ent in order to support the full blown diabetic state This was one of the first observations which illustrated permissive action In animals with a normal pancreas and supply of insulin the administration of an excess *of the adrenal cortical hormones can also cause severe hyper* glycemia and glycosuria Should the diabetogenicity of the adrenal steroid hormones be regarded as a primary action of these compounds themselves or does hypercorticalism simply sensitize or release the in hibition of other physiologic mechanisms which then run wild and cause a diabetic state?

Long I had another question in mind also There are probably a

Long That is the point I was interested in that there is really a curve beginning at a minimal dose below which nothing happens and continuing until with increased amounts more and more happens

Lukens In the case of the cat which has a very high requirement for cortical hormone it was a long while before we could find any hormone that would have a diabetogenic effect in that species The level for permissive action then differs with different species and within a certain range for each species Would that be a fair statement?

Ingle Yes

White The type of stress may also affect in each instance the degree of utilization of the steroid by tissues

Long Would you say in reference to the animals with diabetes that the glycosuria disappears because the supply of adrenal cortical hormone is not adequate to maintain it?

Ingle I suspect that muscle work and exposure to cold cause a decrease in the glycosuria of the diabetic rat because we have introduced a new factor namely an increased utilization of carbohydrate to meet increased energy needs At least some acceleration in glucose oxidation can take place without added insulin

Pitts What are you measuring so far as the total economy of the animal is concerned when you measure whether or not it puts out glucose in the urine? It seems to me that it is a very complicated end point and one that can be influenced in many different ways One way would be the effect of cortisone directly on the kidney Even that is complicated for glycosuria might be effected by changes in filtration rate cellular activity or a combination of both On the other hand are you studying metabolic effect say a diabetogenic action? Are you necessarily always studying the same thing? Might not under one circumstance the appearance of glucose in the urine be due to one cause and under the action of another agent might it not result from entirely different causes?

Ingle This is true We are confronted with the question of whether a state of hypercorticalism can exist in the diabetic organism without causing an increase in glycosuria We do not know that the level of glycosuria is a valid index of either hyper or hypo-corticalism However in our studies on permissive action we have employed several other criteria (2)

Ralli Are you leading us Dr Pitts to the role of the kidney in this situation?

Pitts Yes I think that in studying glycosuria the kidney cannot be disregarded but I readily admit that the kidney is not everything in glycosuria

Long In most cases when you have found glycosuria you have also found that the blood sugar was increased?

also known that adrenalectomized animals on a fixed intake of adrenal cortical hormones adequate to maintain a state of eucorticalism during nonstress conditions become adrenally insufficient during severe stress It can then be expected that certain metabolic adjustments to severe stress which are dependent upon the permissive action of the cortical hormones will drop out because the limited amounts of cortical hormones are no longer adequate to support them We consider it probable that ethylenediamine has an extra adrenal diabetogenic effect which is masked in the adrenalectomized depancreatized rat given a fixed moderate intake of exogenous hormone because the toxic action of ethylene diamine has caused a state of adrenal cortical insufficiency Adrenal cortical insufficiency is averted in the ethylenediamine treated animal having an intact anterior pituitary adrenal cortex axis, for the increased secretion of adrenal cortical hormones maintains a state of eucorticalism which is essential for permissive action

The implications of this hypothesis are important During severe stress it may not suffice merely to maintain a uniform intake of hormone in order to support permissive action In the nonadrenalectomized animal the increased secretory response of the adrenal cortices may be required to support permissive action or in adrenalectomized animals an increase in exogenous hormone may be required to support responsiveness to extra adrenal stimuli

The hypothesis has a bearing on the interpretation of Dr Selye s concepts of the role of the adrenal cortical response to stressors in the etiology of the adaptation diseases I hope that later in the Conference there will be an opportunity to discuss some of his experiments along these lines

Lukens When a rat is on a minimal life maintaining dose of adrenal extract is it then able to have diabetes?

Ingle Not with a minimal life maintaining dose The adrenalectomized rat will continue to live when treated with 0 25 to 0 5 ml of aqueous adrenal cortical extract per day In order to restore responsiveness to a diabetogenic agent the requirement would be about 3 ml of the same extract

Lukens Or six times as much?

Ingle Yes That is about what the animal needs to restore the status quo of eucorticalism under nonstress conditions or mild stress If the adrenalectomized animal is subjected to severe stress larger amounts of exogenous hormone are required to support or permit normal metabolic responsiveness

Lukens The permissive action then can take place within certain dosage limits?

Ingle Yes

Pitts I was thinking purely in terms of the thyroid response to variations in environmental temperature as being rather slow

Ingle Sellers (8) has shown that as far as metabolic response is concerned the thyroidectomized animal shows the response to cold

Lukens Have you ever produced increased glycosuria of a week s duration with any stimulus? I ask in reference to Dr Astwood s question because the length of time it takes for an infection to develop and to produce metabolic effects is such that it might well be missed in the ordinary acute laboratory experiment of one day s duration

Ingle Ethylenediamine is the only severe stressor which exacerbates diabetes in our limited experience We have given it for 10 days Estrogen a mild stressor has been given for 30 days

Astwood A number of people have studied the effect of infections in diabetic rats and as far as I know the result has always been that the diabetes has improved as measured by daily glucose excretion

Ralli Isn t that specific for the rat? The depancreatized dog for example responds differently from the rat to infection

Ingle Have such studies been done on animals given a uniform food intake? I would expect the infected animal to eat less

Astwood I think that is one of the difficulties

Long If a stitch abscess occurs following pancreatectomy or if an abscess develops in the peritoneum the effect on the diabetes is much the same as in the human diabetic In both the cat and the dog incision and drainage of the abscess is followed by a prompt decrease in the severity of the diabetes The rat however appears to be atypical

Lukens Rats are enormously resistant to many infections

White There is I believe a sort of unspoken indication among the discussants that one would generally expect the same kind of response to all types of stress and I think this may relate to Dr Pitts point that the glycosuria is really not only an end point but something measurable at the conclusion of a series of reactions I am sure you would agree Dr Ingle that it would be very surprising if all of these stressors had the same relationship to permissive doses of adrenal cortical extract Not only are different degrees of requirements imposed upon the tissues for the amount of adrenal cortical steroid which is needed to withstand the particular stress which is the point I made before but many of the stressors which you are using have differing effects on other metabolic processes e g cold versus ethylenediamine When the stressors are in the form of agents which not only have a relationship to gluconeogenesis and the end point being measured glycosuria but which also have effects on other processes e g perhaps direct combination with enzyme proteins it would seem to me very surprising if all these stressors were exerting the same influences and operating via the same

Ingle Yes It isn t just a matter of changing the renal threshold

Sayers May I ask whether the blood sugar is decreased in animals that are exercised?

Ingle Yes

Sayers What about the other stresses that you employed for instance cold or ethylenediamine?

Ingle We are studying the effect of ethylenediamine upon the level of blood glucose at the present time These studies show that the in creased excretion of glucose is accompanied by a rise in blood glucose during the administration of ethylenediamine

Astwood I wonder whether we could induce Dr Ingle to discuss the peculiar fact that in the human being an illness particularly an infection makes the diabetes worse while in a rat as he has already mentioned a stress seems to make the diabetes better

Ingle We have not studied the effect of infections in the rat Frac tures which may cause a temporary exacerbation of diabetes in some patients cause a small increase in the glycosuria of the diabetic rat but only for a day or two Nonspecific stressors do not have a uniform effect upon diabetes in the rat Most stressors have no significant effect until the animal is brought near death A few toxic agents such as dilute solutions of formaldehyde cause a significant suppression of glycosuria (6)

Pitts Have you tried the stress of increased thyroid activity brought about by the administration of thyroid hormone?

Ingle We have not studied the effect of thyroid hormone upon gly cosuria According to Houssay (7) the administration of thyroid causes some exacerbation of diabetes We have shown that the effect of thyroxin upon nitrogen balance is independent of adrenal cortical function

Glycosuria is not the only criterion that we have used in studies of permissive action We have used hair growth work performance and in several studies nitrogen balance as a criterion The adrenalectomized rat treated with adrenal cortical extract shows a normal catabolic re sponse when exposed to cold

Pitts Why does it seem odd to you that the response to cold should be rather slow and that the return to normal should also be slow It would appear to me an eminently reasonable thing

Ingle I had expected that the change in glycosuria would be directly related to the energy requirement of the animal falling abruptly when the animal was exposed to cold and rising abruptly when the animal was returned to room temperature The response of the glycosuria level to periods of exercise and rest is very prompt whereas the response to and recovery from exposure to cold is slow What is your basis for expecting the slow response to cold?

eosinopenia It is obvious that if these other substances are mobilized the overall eosinophil response will be enhanced

Loewi Do I understand you to say that with epinephrine an eosinopenia can be produced after adrenalectomy?

Long Is that a matter of relative dose?

Thorn It can be done very easily with 0 3 to 0 5 mg of epinephrine

Long I thought that at one time you published several papers in which you said you did not get it with 0 25 milligrams

Thorn The majority of patients with Addison s disease did not get a significant eosinopenic response with 0 3 mg injected subcutaneously Epinephrine given relatively rapidly intravenously is an effective means of producing an eosinopenia but if the same dose i e 0 3 or 0 5 mg is spread over a period of four hours rather than given within a period of 30 minutes one may not observe an eosinopenia Thus the eosinopenia in a patient with or without his adrenals would appear to depend upon epinephrine attaining a threshold dosage

Long Isn t that contrary to the work of the Finnish investigators (9) who infused epinephrine over long periods and found an eosinopenia?

Thorn All I can say is that it appears to us that in the same individual a dose of epinephrine is more effective in regard to eosinopenic action if given more rapidly

Ralli Has it anything to do with the blood flow?

Thorn No

Gellhorn I wonder whether the fact that epinephrine acts more effectively when given in a short time is an indication that a central action is involved whereas in the case of ACTH a peripheral action is involved

Thorn There is an interesting comparison between the two hormones In the case of epinephrine it appears to be a threshold discharge phenomenon whereas with ACTH time and a build up reaction is necessary to obtain a maximum response For instance one unit of ACTH given over a four hour period gives a small but definite increase in 17 hydroxysteroid excretion whereas one unit of ACTH given as a twenty four hour continuous intravenous infusion may cause a very marked degree of adrenal activation Regarding epinephrine no one to my knowledge has demonstrated by hormonal assay methods any activation of the adrenal cortex with epinephrine in man

Gellhorn I wonder whether I understood both Dr Pitts and Dr White correctly that the concept of nonspecific stress has been useful in the investigation of these problems involving the adrenal cortex? In a way I believe that the concept of nonspecific stress is a prescientific concept Specificities seem to be involved in every one of these stresses and what has been emphasized are certain common factors or common

mechanisms even though they affected the end point, glycosuria to the same degree

Ingle The only reason for expecting nonspecific stressors to affect glycosuria in the same manner arises from Dr Selye s emphasis upon the uniformity of the pattern of response to different kinds of stressors and emphasis upon the role of adrenal cortical activation in mediating the metabolic responses to stressors We and others have found wide differences in some metabolic responses to stressors and have shown that the exciting mechanisms for some responses to stressors are extra adrenal

Long If an animal is given a constant dose of adrenal cortical hor mone and subjected to cold does his eosinophil count fall? This could be used instead of the glycosuria as an indication of adrenal cortical activity

Ingle We have not studied changes in eosinophils

Sayers Has an eosinopenia been observed in adrenalectomized ani mals on a constant dose of cortical hormone after cold exposure?

Long I was wondering whether anybody here had done this experi ment The eosinopenia obviously stems from the so called permissive action that is the lytic effect if it is a lytic effect on eosinophils which is usually said to be due to a rising content of adrenal cortical hormone in the blood If the amount of adrenal cortical hormone in the blood is kept constant by injection and then a stressing agent is applied what happens to the eosinophils? Does the eosinophil count provide a better index of the effects of stress than glycosuria? Will it always respond the same way?

Ingle There are certain stimuli which will cause eosinopenia in adrenalectomized patients and patients with Addison s disease but I read a manuscript the other day in which the author insisted that this happens only when functional cortical tissue remains

Thorn I would take exception to that

Ingle Many people agree with you

Thorn If a given patient fails to respond to an infusion of ACTH which is the case in patients with Addison s disease and patients in whom we have carried out bilateral complete adrenalectomy and if in the same individual an eosinopenia can be easily induced by administer ing 0 3 to 0 5 mg of epinephrine it does not seem plausible to account for the eosinopenia on the basis of adrenal steroid mediation On the other hand the most potent stimulus for eosinopenia in the presence of adequate adrenal cortex is the exogenous administration of ACTH In view of this I feel that we must assume that there are other sub stances and other mechanisms in the body which independently of the adrenal cortex or adrenal cortical steroids are capable of producing an

tomized In this respect the experimental animal differs considerably from our studies on intact man

Long That is not what you said before You said there was no evidence

Thorn There is no direct evidence to date in man of adrenal cortical activation with epinephrine

Lukens If the tissues use up all the excess adrenal hormone which is put out it is the same thing

White No it is not the same thing

Lukens Correct but it might have the same outward appearance to one measuring urinary steroids

Vogt In man it is difficult to keep up what appears to be an increased output of cortical hormone due to epinephrine for any length of time That may possibly explain why there is not increased excretion let us say of metabolites or even cortical hormone in the urine Man is more difficult to study in this regard

Thorn All of our experiments have been carried out with four hour fractionations and occasionally with two hour fractionations of the urine Thus I doubt whether we would miss a temporary increase in secretory activity Certainly the eosinophil count is down at the time that the urinary assay is carried out

Vogt But certainly the eosinophils take time to go down don t they?

Thorn It is true that the eosinophils do take time to achieve their maximum fall but we have shown that when a fall of 50 per cent is attained with ACTH an increase in urinary steroids can be detected during that period very easily

Vogt But much of the cortical hormone must be being wasted

Thorn No there is not much excess of hormone in the range of eosinophil fall between 40 and 80 per cent It is only over a 90 per cent fall in eosinophils that we would feel that an excess of hormone might be secreted which would not necessarily be reflected in the eosinophil fall but as a greatly increased urinary excretion of steroid

Vogt How do you administer the hormone?

Thorn The cortical hormone in these experiments is always given intravenously We usually employ a constant intravenous infusion

Vogt When you give it by single injection certainly a lot is wasted by simple spilling over into the urine

Thorn With a sudden large intravenous infusion of hormone of course there would be an excess available and there might be appreciable spilling over into the urine It would seem to me that studies in intact man in which the hormone was given either as a sudden single intravenous injection or as a continued sustained intravenous injection would match either of the most likely possibilities which would occur when the gland itself was stimulated

effects Nevertheless, every stress group may still involve specific dif ferences

Pitts I had in mind the fact that cold and exercise might be inter preted physiologically as having an effect say, on glucose utilization and would lower blood glucose that way Concerning other stresses, I have no idea what the effects on glucose utilization glycogenolysis, or gluconeogenesis might be or on tubular reabsorption or on any of the other possible mediating factors There might be an element of specificity in some of these stresses, and yet all of them might lead to the same general end in different ways Or they might lead to entirely different ends I don t think they are as nonspecific as implied

Long Getting back to Dr Thorn s remark that there has not been any demonstration that epinephrine stimulated the adrenal cortex is not the fall in eosinophils in a man given epinephrine indicative of secretion of adrenal cortical hormone?

Thorn As my previous discussion indicated an eosinopenia follow ing epinephrine is not in itself conclusive evidence of adrenal cortical steroid discharge

Long Putting aside for the moment that a prolonged infusion of epinephrine will not alter the eosinophil count and taking the doses that you first proposed in man 0 25 mg given to a normal individual which was followed in four hours by a 80 or 90 per cent drop in eosino phils was that not specific and had that nothing to do with the stimu lation of the adrenal cortex?

Thorn Three laboratories working independently have been unable to demonstrate that epinephrine administered to man stimulates the adrenal cortex to increase its output of adrenal steroid as measured in one laboratory by blood hormonal levels and in two laboratories by urinary excretion of hormones From these data I think that at pres ent we are not justified in assuming that the eosinophil fall following epinephrine necessarily parallels adrenal cortical activation It would appear to be a completely independent phenomenon

Long This demonstrates then that man is very different from the dog I don t have to speak for Dr Vogt but certainly her experiments clearly demonstrated that if epinephrine is given in the physiologic range certainly within a few minutes there is a marked increase in the output of adrenal cortical steroids What you are saying in effect is that the human pituitary system does not respond to epinephrine

Thorn All I can say is that in man we have been disappointed in not being able to confirm the observations made in animals It is to be noted however that in Dr Vogt s experiments as she has explained epinephrine did not lead to an increase in output of adrenal cortical hormone from the adrenal gland until the animal was splanchnicec

that in man at least epinephrine is a potent mechanism for producing the change in these circulating cells

Ralli Do you feel that the uric acid creatinine ratio gives any objective evidence of adrenal stimulation?

Thorn In short term experiments I feel that changes in urinary uric acid and creatinine ratio are of very limited value in the interpretation of adrenal cortical response

Vogt If cortical hormone or ACTH is injected into a patient who is not exposed to stress and therefore so to speak doesn t need the extra dose there are signs of excretion in the urine Do you think that this means that when the same person produces cortical hormone in response to stress the same amount will appear in the urine? May it not be metabolized differently and therefore not be found?

Thorn Such experiments are now in progress We have demonstrated the fact that an increase in urinary steroid excretion can be reliably shown following the continuous intravenous administration over a period of four hours of one to two units of ACTH i e 0 25 to 0 50 units per hour The experimental design which we are employing is to test the individual s sensitivity to this dose of ACTH study the individual s response to exposure to cold for a four hour period and then add ACTH to the exposure to cold to detect whether the ACTH stimulation under these circumstances gives rise to the added quantity of urinary hormone which we are able to detect in the absence of the cold

Vogt Would it not be clearer if instead of using 0 25 units of ACTH you were using a small dose of cortical hormone itself?

Thorn No If one is using intact individuals ACTH I believe is preferable at this dose level The spontaneous involution of the adrenal which occurs with 11 17 oxygenated steroid administration makes the interpretation of urinary changes unreliable in individuals with intact adrenals given small doses of steroid This of course does not obtain when massive hormone dosage is employed

Long So many papers are written nowadays in which the criterion of lack of or increased activity of the adrenal cortex is based on eosinophil counts I don t need to remind this group that when epinephrine is given or when an animal is subjected to stress which causes it to release epinephrine one of the things that happens is splenic contraction Certainly in man as the Finnish investigators and many others have shown the first effect of epinephrine may be a 100 per cent increase in the eosinophil count Thus there is a high degree of uncertainty as to the extent of the initial rise even before the eosinophil count begins to fall so that under some circumstances when a second measurement of eosinophils is made it is found to be back at the original level and that is interpreted as a lack of adrenal cortical activity

Vogt According to Colfer de Groot and Harris (10), intravenously administered epinephrine does not cause a fall in peripheral lymphocytes which is a similar type of test If Dr Thorn s interpretation of his observations in man is right, it may be that man behaves like the rabbit and not like the dog

To come back to the question of the possibility of using in animal experiments, the determination of the eosinophils in the same way as Dr Ingle uses the glycosuria there is one difficulty at least where mice are concerned Apart from epinephrine, with which I have had no experience there are certain substances which will cause a fall in eosinophils in adrenalectomized mice For example very small quantities of dog s plasma globulin will cause a fall in eosinophils in the completely adrenalectomized mouse Therefore there must be at least two mechanisms which can cause in the mouse a fall in eosinophils, one being the release of cortical hormone and the second some mechanism which can be stimulated for instance, by the injection of dog globulin

Thorn I believe that the fall in circulating eosinophils correlates extremely well with adrenal cortical hormone output following exogenous ACTH stimulation However it is evident that since factors other than the adrenal cortex may induce the eosinopenia noted in stress it is of limited significance in terms of interpreting the participation of the adrenal cortex On the other hand a failure to observe an eosinopenia is a considerable help clinically as it suggests the absence of significant adrenal cortical activation at that time Thus it would appear that in evaluating the capacity of the adrenal to respond to exogenous ACTH the eosinophil response is extremely helpful whereas the overall eosinophil change in the reaction to stress does not necessarily prove to be a true indicator of adrenal cortical participation

Ralli Are you suggesting that if an individual is exposed to cold stress an eosinopenia will be provoked? Short term exposure to cold is notably unpredictable in its effect on the excretion of the cortical steroid and I believe that in man consistent changes in the steroid excretion do not occur

Thorn I agree with that

Ralli You can t say that that doesn t prove the adrenal is not stimulated

Thorn That is correct but one cannot say that the adrenal has been stimulated if one is not able to demonstrate either an increase in circulating hormone level or the excretion of an increased quantity of urinary steroids Although it is undoubtedly true that exposure to cold increases the adrenal cortical activity one is not justified in stating this categorically on the basis of an eosinopenia since we have demonstrated

brain extracts also cause a fall and that this is due to their springomyelin content

Thorn I believe Dr David Hume showed that using specially pre pared hypothalamic extracts

Long Yes The point I am trying to get around to is that using any brain extract not necessarily from the hypothalamus would do it

White In your experiments with epinephrine Dr Thorn in the human have you followed lymphocytes? Does one get a lymphopenia in the adrenalectomized patient with epinephrine?

Thorn It is more difficult to measure significant changes in lympho cytes in man but the direction of change is similar to that of eosinophils It is our interpretation that this is due to the much more rapid turnover of lymphocytes and the larger reservoir available

White Certainly in the mouse the lymphophenia with epinephrine does not occur if the animal is adrenalectomized

Thorn I cannot give any figures on this in man

White We are focusing a good deal of attention perhaps too much attention on a common specific pathway for stresses via the adrenal cortex I wonder whether a true measure of what stress does physi ologically isn t better reflected in the adrenalectomized animal?

Pitts Dr Ingle as a point of information is there such a thing as nonspecific stress in an adrenalectomized animal? Is the nonspecificity casually related to the response of the adrenal cortex?

Ingle In Dr Selye s concept of the alarm reaction he has empha sized the similarity of the pattern of certain biochemical physiologic and morphologic changes which ensue irrespective of the nature of the stressor The adrenal cortex is considered to be activated during exposure to any severe stressor and has been considered by Dr Selye and many others to mediate some of the biologic consequences of exposure to stressors There is no doubt about the nonspecificity of noxious stimuli which will elicit the alarm reaction but perhaps the similarity of responses to different stimuli and the role of the adrenal cortex in mediating the various responses making up the alarm reaction have been overemphasized

White One piece of evidence that stressors do different things and that they don t all act via the adrenal cortex is seen in the extent and magnitude of the lymphocytosis which can be produced in an adrenal ectomized animal by varying kinds of stress The striking effect is one of tremendous lymphocyte production in the absence of the restraining influence of the adrenal cortex and yet the degree to which various stressors do this will differ quite markedly from one to another What the mediating mechanism is I am not certain but just as there is a difference in the rate of glucose utilization depending on the type of

I don t think one is entitled to say that either In our splenectomized rats the eosinophil count begins to fall soon after epinephrine injection but in the intact rat, there is initially a large rise

Thorn That is true for epinephrine It certainly would not be true for the administration of crystalline steroid as there one observes a continuous fall in eosinophil level

Long Yes I am speaking of those circumstances cold and so on in which there is a release of epinephrine and autonomic discharge

Thorn To summarize our experience the absence of eosinopenia following an initial exposure to a stress situation is strong indirect evidence against adrenal activation On the other hand one is not justified in attempting to interpret quantitatively adrenal cortical response to stress on the basis of the eosinopenia alone since this reaction may be induced by factors other than the adrenal steroids liberated during the exposure to stress

Long I would not disagree with that It has already been said in other places that there are uncertainties regarding the eosinophil count

Thorn I don t believe it is a question of uncertainty regarding the eosinophil count but a question of capacity to interpret the changes which are measured

Bush It is surely no more logical to say that the absence of eosinopenia is definitely correlated with the absence of adrenal cortical stimulation in the light of present knowledge than it is to say the reverse Until we have investigated whether there are any agents which can cause a rise in eosinophils we cannot assume that the absence of a fall indicates an absence of adrenal cortical stimulation

Loewi How did you get to the idea to check globulin?

Vogt In work carried out by Dr Bibile and myself we were using eosinophil counts in adrenalectomized mice or trying to use them in order to test for cortical hormones in blood We ran up against a difficulty in using dogs blood in that quite small quantities of arterial dog blood contained something which caused a fall in the eosinophils From a quantitative point of view it was impossible that this effect was due to cortical hormones because it would have meant that there were incredible quantities of cortical hormones in the arterial blood We started fractionations and found the globulin fraction active We haven t analyzed the effect further

Loewi You did not use any other globulin?

Vogt We have tried a few different species It was only the dog which produced it regularly or gave rather large falls Occasionally it happened with the cat and the human but mouse rat guinea pig and fowl plasma did not cause it

Long I have heard that somebody in Great Britain has shown that

Li What is the exact dose?

Vogt The dose corresponds to 0 1 ml plasma After it is fractionated, I don t know what it corresponds to but it is not very much just the globulin contained in 0 1 ml dog plasma It would have to be a very potent steroid if the amount contained in the globulin fraction of 0 1 ml plasma suffices It might of course be any substance associated or precipitated with the globulin *but certainly it cannot be extracted* with an organic solvent as can ordinary steroids That is all I can tell It may be a steroid which is unextractable

Ralli Does this response occur with plasma from any number of dogs?

Vogt There is a slight variation in the intensity A particular plasma has the same activity on different batches of mice but different dogs plasma have a different degree of activity Plasma from one animal may be potent in a smaller dose than the plasma from another animal There is variation from dog to dog

Ralli Does it have any relation to species?

Vogt That I don t know

Lukens Does it have any relation to the preceding stimulus of the dog?

Vogt Well the blood was collected either by venipuncture in the conscious dog or by arterial bleeding under anesthesia We did not try to correlate the effects with the conditions under which the blood was obtained

Thorn Favour has shown a very good *in vitro* lympholysis with human plasma Eosinopenia cannot be demonstrated *in vitro* but a lympholysis can However it never seemed to me that the amounts of plasma involved were large enough to account for that effect on the basis of the steroid hormone although one cannot say for sure

Vogt If globulin is prepared from plasma by dialysis the dialysate is the ineffective part and what is left behind is active If it is a steroid of some sort it must be very firmly associated with the protein It is not dialyzable or extractable with an organic solvent

Bush Isn t it unlikely that it is a steroid because it is inactive in the cold exposure test?

Vogt Well *it is certainly not an adrenal steroid but there may be* something else which acts on eosinophils

Bush You mean it might be a trace of cortisone or something else

Ralli Could this be an allergic reaction of some kind?

Vogt If it is it could be only one of those inborn allergic reactions for which preceding sensitization is not necessary That is of course quite possible That is why Dr Bibile who did these experiments tried all sorts of things hoping to find something else which would produce

stress so there could very well be a difference in the rate at which lymphocytes are utilized

Long Dr Pitts and Dr Ingle s last remarks lead to another point which is in line with what we have been talking about that is the emphasis which has been placed on the release of ACTH from the pituitary in response to stress At times, the literature seems to indicate that this is the only thing which happens but is this true? When the animal is placed in the cold as Dr Pitts has pointed out there is also thyroid activation We have very little information as to how rapidly that occurs but there is some work to indicate that it may be almost as fast as the response of the adrenal cortex I believe that trying to assess the response to cold or to any of these stressful circumstances merely in terms of the adrenal cortex is probably misleading indeed because we know that in many circumstances ultimately the other endocrine glands are involved In some circumstances, there is gonad atrophy in others, hypertrophy of the thyroid along with hypertrophy of the adrenal

Li Is it possible also that there are different forms of ACTH and that different stresses might cause the secretion of different types of ACTH? For the past two months we have been trying out the effects of different preparations of ACTH on normal and hypophysectomized rats with respect to eosinopenia We have one preparation which has a very marked adrenal ascorbic acid depleting activity as well as adrenal weight increasing potency but which produces no eosinopenia when it is injected into hypophysectomized or normal rats There was found no correlation between the eosinopenic activity and the adrenal ascorbic acid depleting activity among various fractions In fact reduction of the adrenal ascorbic acid depleting activity by NaOH heat treatment does not inactivate the eosinopenic activity which suggests that eosinopenic activity might be a separate factor

Long How many kinds of ACTH would that make?

Li We don t know exactly how many different ACTH s there are I was merely talking about ACTH activities

I should like to ask Dr Vogt about the globulin A possible explanation might be that there are adrenal steroids associated with the globulin which is causing the eosinopenia in the adrenalectomized mouse Or have you tried using different kinds of protein?

Vogt Yes we have tried a number of proteins several albumins including ovalbumin and they were all ineffective I think it is practically impossible that there are steroids in the globulin fractions At least if there are they are not extractable with any organic solvent It must be a very queer sort of combination and they would have to be there in very large quantities because the total amount of globulin required is extremely small

MECHANISMS THROUGH WHICH THE ADRENAL CORTEX PRODUCES QUALITATIVFLY DIFFERENT EFFECTS*

HANS SELYE

Institute of Experimental Medicine and Surgery
University of Montreal

I HAVE NOT ENTITLED my opening remarks The plurality of adrenal hormones particularly since Dr Vogt is to speak about new work on the so called natural mineralo corticoid or salt active hormone I merely plan to survey the evidence which suggests that the adrenal cortex can produce qualitatively distinct effects quite irrespective of whether different hormonal substances are produced

In order to outline the ways through which the adrenal cortex might produce qualitatively different syndromes may I first present in Figure 1 the now familiar sketch on endocrine correlations during stress Before discussing the interrelations depicted in Figure 1 I should like to define my views concerning three points because these are often misinterpreted in the current literature and hence have given rise to much confusion

STRESS IS BY DEFINITION ALWAYS NONSPECIFIC

1) In earlier writings (1 2) I defined stress as the sum of all nonspecific biologic phenomena (including damage and defense) It may be localized (as exemplified by inflammation) or systemic (as exemplified by the general adaptation syndrome) Those who speak of various kinds of stresses evidently do not use the term according to this definition yet I am not aware of any alternative definition

A stressor is an agent capable of producing stress I distinguish the systemic stressor or alarm stimulus (an agent capable of producing a general adaptation syndrome or G A S) from the topical stressor (an irritant which causes local *nonspecific tissue changes e g inflammation or necrosis*) Naturally infections cold drugs etc produce qualitatively different effects because apart from acting as stressors they also possess specific properties of their own The latter are super

*These investigations were made possible in part due to grants received from the National Research Council of Canada the National Cancer Institute and the Canadian Arthritis and Rheumatism Society

the same reaction Horse serum and an extract of Ascaris and so on were tried but nothing had the same results

Long I still have a long list of questions here but I think in the interest of our conversations tomorrow we shall stop at this point

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THE SO CALLED PERMISSIVE ACTIONS OF CORTICOIDS ARE BUT ONE OF THE PHENOMENA OF CONDITIONING

3) Not only hormones but stress dietary constituents and many other agents can act as conditioning factors These have been defined as factors which have little or no activity in themselves but can significantly alter a response to a stimulus For instance sodium can act as a conditioning factor of DCA activity stress which in the absence of the adrenals manifests itself by hypoglycemia can so condition the actions of gluco-corticoids that even virtually inactive threshold doses of these will produce marked hyperglycemia

It is largely a matter of one s point of view whether in any such instance the hormonal or the nonhormonal agent is designated as the conditioning factor It would be equally correct to say that the DCA *effects are conditioned by sodium or that the sodium effects are conditioned* by DCA The important point is that when corticoids are administered or endogenously produced at ineffective levels they may nevertheless produce manifest effects by conditioning the body to certain stress responses For example they may reverse hypo- into hyperglycemia It is for this particular instance that Dr Ingle if I understand him correctly proposes the term permissive action This strikes me as entirely acceptable since here the hormone does not so much produce an effect of itself as it conditions the body s response in such a manner that the effect of another agent is permitted

To return to a discussion of Figure 1 although each stressor (cold infections nervous strain) has a variety of specific actions of its own which may condition the response of individual target organs during *stress these specific actions are not illustrated The drawing does show* however that the stressor acts upon the target the body or some part of it directly (thick arrow) and indirectly through the pituitary and adrenal Through some unknown pathway (question mark) a stimulus travels from the directly injured target area to the anterior pituitary It notifies the latter that a condition of stress exists thus inducing it to discharge ACTH It is quite possible that this first mediator of hormonal defense is not always the same In some instances it may be an epinephrine discharge in others a liberation of histamine like toxic tissue metabolites a nervous impulse or even a sudden deficiency in some vitally important body constituent such as glucose or an enzyme

ACTH stimulates the adrenal cortex to discharge corticoids Some of these the mineralo-corticoids or prophlogistic corticoids (P C) stimulate *the proliferative ability and reactivity of connective tissue* By enhancing the inflammatory potential they help to put up a strong barricade of connective tissue through which the body is protected

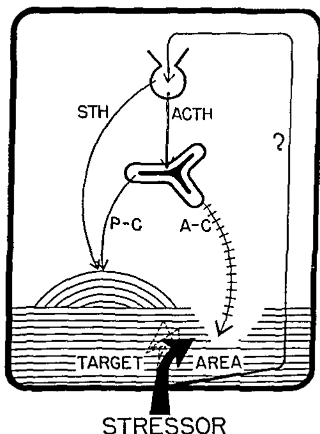


FIGURE 1 Schematic outline of interrelations between the stressor and the hormonal defense system

imposed upon their stressor actions and may condition the body's response modifying certain manifestations of the basically stereotyped and always nonspecific stress reaction

STRESS DEFINITELY OCCURS EVEN IN THE ABSENCE OF THE HYPOPHYSIS AND/OR THE ADRENAL CORTEX

2) Indeed some of our earliest experiments on the adaptation syndrome were designed to demonstrate that certain characteristic manifestations of stress (e g hypoglycemia hypochloremia) are rarely evident except in hypophysectomized or adrenalectomized animals in which the normal adaptive response of the hypophysis adrenal system is eliminated

transformations of a single secretory product into qualitatively different hormones must also be considered

Our latest experiments suggest that even the distribution of circulating corticoids in various organs or their selective fixation in certain tissues may result in qualitatively different effects It is obvious that if for some reason an organ has a selective affinity for a corticoid this hormone can act on such an organ selectively even without any change in blood level or urinary titer This may be a far fetched possibility at the present time since such selective hormone fixation has not yet been demonstrated to occur for endogenously secreted corticoids It has however been shown that estrogens and thyroxin can be selectively concentrated in certain tissues Furthermore experiments have demonstrated that when artificial fixation of hormones in the skin is achieved by a special experimental technique the local response is greatly exaggerated

This theoretical introduction is somewhat lengthy and complicated However I believe it is indispensable to formulate the problems clearly before attempting to interpret factual observations concerning the plurality of the mechanisms through which corticoids may exert qualitatively different actions

Figure 2 illustrates the organs of two rats which have been treated with exactly the same amount of desoxycorticosterone acetate namely 10 mg per day subcutaneously during a period of 53 days The organs on the left show manifest signs of the so-called DCA encephalopathy (with brain edema) cardiac hypertrophy enlargement of the kidney (with nephrosclerosis) and periarteritis nodosa of the mesenteric vessels Most of the animals in this group died as a result of hypertensive changes The organs on the right come from a rat which was treated with exactly the same amount of DCA and yet none of the typical changes are manifest The brain heart kidney and the mesenteric vessels are macroscopically of normal appearance and this was confirmed by microscopic examination All the animals in both these groups were specifically sensitized to the toxic manifestations of DCA overdosage by unilateral nephrectomy and by a 1 per cent NaCl solution given as drinking water The protection of the animals represented on the right side of the picture was accomplished solely by hypophysectomy In other words ablation of the pituitary appears to render the rat resistant to the typical hypertensive and rheumatic like changes induced by DCA This protection is evident despite sensitization by such conditioning factors as unilateral nephrectomy and a high sodium intake

On the basis of Figure 1 one would be tempted to assume that the toxicity of the prophlogistic corticoid in this case DCA depended largely upon a pituitary factor perhaps STH Here again in a certain

against further invasion by the pathogenic stressor agent However under ordinary conditions, ACTH stimulates the adrenal cortex much more effectively to secrete the so called gluco corticoids or anti phlogistic corticoids (A C) These inhibit the ability of the body to put up granulomatous barricades in the path of the invader in fact they tend to cause involution of connective tissue with a pronounced depression of the inflammatory potential Thus they open the way to the spreading of infection

As far as is known ACTH always stimulates the adrenal cortex to produce the various corticoids in the same proportion and always with a great predominance of A Cs However the somatotrophic hormone (STH) of the pituitary also increases the inflammatory potential of connective tissue somewhat as the P Cs do, hence, it sensitizes the target area to the actions of the latter

It is possible that the hypophysis also secretes some special corti cotrophin which induces the adrenal to elaborate predominantly P Cs indeed STH itself may possess such effects although this has not yet been proven In any event even if ACTH were the only corticotrophin the actions of the corticoids produced under its influence could still be vastly different depending upon conditioning factors such as STH which specifically sensitize the target area for one or the other type of corticoid action Actually perhaps conditioning factors could even alter the response to ACTH of the adrenal cortex itself so that its cells would produce more A Cs or P Cs Thus during stress one or the other type of effect could predominate

The fundamental reaction pattern to topical stressors is inflammation to systemic stressors the general adaptation syndrome Various com binations of these two basic responses constitute the essence of most diseases

Qualitatively different corticoid effects may arise during stress due to differences in the conditioning of the response at various levels particu larly in the target area the hypothalamo pituitary system and the adrenal cortex For instance an agent may in addition to its stressor effect have certain specific properties which alter the response of the target area directly The hypophysis may respond during stress by the production of STH and ACTH in different proportions indeed it may produce qualitatively different corticotrophins although this has not yet been definitely proved The adrenal may respond to the same ACTH by the secretion of the various coritcoids in different proportions due to factors conditioning its own response to ACTH The target organ response to any one corticoid may be altered by other corticoids by STH by sodium and by many other factors In connection with a plurality of actions aris ing at this last peripheral level it is well to remember that metabolic

sense we might speak of an indispensable hypophyseal conditioning for the toxic DCA actions

The fact that such lesions cannot be produced in hypophysectomized rats by DCA has been shown before (3) and yet it could not be concluded that the pituitary was necessary for all the actions of this hormone * Among the other usual DCA effects the polyuria was actually aggravated the hypochloremia was abolished while the enlargement of the kidney seen in intact DCA treated rats was merely diminished not abolished by ablation of the hypophysis Apparently hypophysectomy influences the various effects of this steroid in different ways leaving some uninfluenced increasing others and abolishing yet others This is what I have called selective conditioning of various targets

Recently my associate Dr Ernesto Salgado and I repeated and confirmed this work on a large number of hypophysectomized rats In this series we observed furthermore that the pressor effect of DCA was only slightly reduced by hypophysectomy although the above mentioned cardiovascular and renal changes were virtually completely blocked We also demonstrated that even simultaneous treatment with DCA and cortisone which in our experience is particularly damaging to the kidney of intact rats caused no nephrosclerosis after hypophysectomy (5) I mention these experiments on hypophysectomized animals to illustrate how the total picture of DCA intoxication can be qualitatively altered by the absence of hypophyseal hormones

Figure 3 illustrates another type of selective conditioning On the left is the heart kidney and adrenal of a normal female control rat On the right are the corresponding organs of a rat of the same size and age This animal received heavy doses (20 mg twice daily) of lyophilized anterior pituitary (LAP) and of DCA (5 mg daily) seven months prior to autopsy during a period of twelve days Both animals originally weighed 106 grams and both were sensitized to mineralo-corticoid actions by unilateral nephrectomy and a 1 per cent NaCl solution as drinking fluid Twelve rats were subjected to the transient hormone overdosage Upon cessation of both hormone and NaCl administration they appeared to be in perfect health yet all but this one animal died during the ensuing seven months with marked nephrosclerosis hypertension periarteritis nodosa and cardiac nodule formation The adrenals were essentially normal This last survivor also shows cardiac and aortal hypertrophy fibrous nodules in the heart (note the white

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* Attention is called here to a misprint on page 513 in the first printing of *STRESS* (4) The line referring to these DCA experiments (3) reads typical hyalinosis can be produced by this mineralo-corticoid compound in the absence of the pituitary it should have read typical hyalinosis cannot be produced

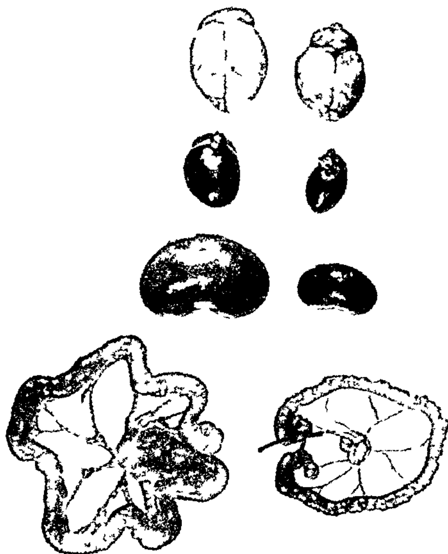


FIGURE 2 Inhibition of certain mineralo corticoid overdosage effects by hypo physectomy Reprinted by permission from Selye H and Horava A *Second Annual Report on Stress* Montreal ACTA Inc 1952

metacorticoid hypertension may be an appropriate designation for this phenomenon in analogy with the expression metahypophyseal diabetes used by Professor Houssay for that type of glycosuria which develops after interruption of pituitary extract treatment

In an informative systematic study of what he has called post DCA hypertension Green *et al* (8) called attention to the most important fact that this type of hypertension exhibits a striking similarity to the essential hypertension occurring in man

From the point of view of selective conditioning our observations are of special interest since they show that the time factor in itself can dissociate the usual manifestations of DCA intoxication and thus qualitatively alter the total picture of corticoid overdosage During the metacorticoid hypertension the characteristic rise in blood pressure the cardiovascular changes and the renal changes typical of DCA over dosage are evident but other manifestations for instance the com pensatory atrophy of the adrenal cortex are absent

Ingle Does the animal receive the high sodium load throughout the time it survives?

Selye In this particular group the sodium treatment was interrupted for a few weeks given again for a few weeks and so on just to show that hypertension and polyuria are aggravated in an animal sensitized by temporary DCA and LAP overdosage by a high sodium intake I believe Dr Green found that sodium does not aggravate metacorticoid hypertension but the differences may be due to the fact that we used a slightly different experimental arrangement and in any case this is not relevant to the subject under discussion

Ralli Why did you unilaterally nephrectomize the animals?

Selye The experiment was primarily designed to show the briefest possible period of heavy hormone overdosage that could cause meta hormonal hypertensive changes at a later time Unilateral nephrectomy and a high sodium intake are not indispensable for the production of such changes but these conditioning factors greatly facilitate their development

Li These animals were not hypophysectomized?

Selye That is right

Li Did you examine the pituitary in these animals?

Selye With ordinary hematoxylin eosin sections nothing noteworthy was seen

Astwood The animal at the right in Figure 3 shows the state at the end of 12 days?

Selye No That is seven months after commencement of the treat ment Both animals were unilaterally nephrectomized at the same time and when one got salt the other also got salt The only difference



FIGURE 3 Meta hormonal changes in the heart and kidney after temporary overdosage with DCA and LAP Reprinted by permission from Selye H *First Annual Report on Stress* Montreal ACTA Inc 1951

spots in the center of the ventricle) and a granular nephrosclerotic kidney Throughout the entire length of observation all these animals *invariably developed acute exacerbations of their hypertension* whenever they were placed on 1 per cent NaCl for a few days The experiment illustrates that a brief temporary excess of adaptive hormones can cause a progressive and eventually fatal disease although at the time of manifest illness there need no longer be any hypophyseal or corticoid hormone excess (1)

Some former members of our laboratory Professor Sidney Friedman (6 7) in Vancouver B C and Dr Leal Prado* in Sao Paulo Brazil had previously and independently observed that even temporary overdosage with DCA alone may cause a hypertension which persists after interruption of hormone therapy Hormone treatment must be continued much longer however to produce a lasting change The term

*Prado J L *Estudos sobre hipertensao hormonal experimental* These apresentada à Escola Paulista de Medicina Sao Paulo (1950)

between the two is that the one on the left (as did all animals in this group) remained otherwise untreated while that on the right received hormone treatment during the first 12 days

Vogt Did they all react the same way in that group?

Selye Yes In the group given hormone treatment 12 out of 12 died and all of them showed lesions There was scatter only in the sense that not all the animals died at the same time

Figure 4 illustrates an experiment which was made possible through the help of Dr Astwood who supplied us with a sample of his oxycellulose purified ACTH At the top is the adrenal and kidney of an untreated control rat Neither this nor the two other animals illustrated were sensitized by partial nephrectomy or by a high sodium diet In the middle are the adrenal and the kidney of a rat which received Dr Astwood s oxycellulose ACTH preparation over a period of 25 days subcutaneously twice daily in oil The daily dose level was initially 0 2 mg and was then gradually raised in the course of treatment to 0 4 mg and finally to 0 6 mg During this time the rats did not grow because of the catabolic and growth inhibitory effect of ACTH Their adrenals became very large they lost their lipid granules and were extremely hyperemic Histologically there was intense dilatation of all the sinusoids in the cortex so that the organ assumed an almost spongy or cavernomatous appearance

Other animals of which a representative is illustrated by the organs in the bottom row received the ACTH treatment mentioned above but at the same time they were treated with an STH preparation kindly supplied by the Armour Company of Chicago The dose of STH was purposely always so adjusted as to maintain a normal growth rate despite the ACTH Consequently the animals in the third group were of the same size at the end of the experiment as the controls Despite this as can readily be seen the kidney in the rats treated with STH plus ACTH was considerably enlarged Histologically it showed signs of hypertrophy and hyperemia but no nephrosclerosis From a functional point of view it is also interesting to note that in this third group which received treatment with both pituitary hormones a pronounced polyuria developed

The most striking finding of course was the enlargement of the adrenal in all the rats of the third group The weight of the adrenal was above the maximum weight observed in any of the rats receiving ACTH alone It is tempting to accept such an experiment as definite proof of some corticotropic action of STH but to my mind the evidence is still not quite convincing It is quite possible that some metabolic change produced by the growth hormone outside of the adrenal so conditioned the gland that it responded differently to ACTH even if the STH itself had no effect on the adrenal at all

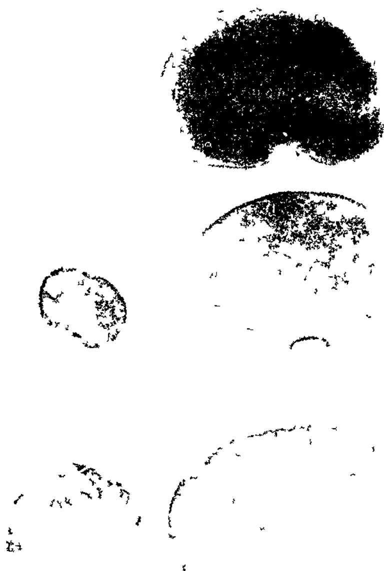


FIGURE [illegible]. Adrenals and kidneys of a normal control (top), an ACTH-treated (middle) and a simultaneously ACTH- and STH-treated (bottom) rat.



FIGURE 5 Effect of STH and stilbestrol given alone or in combination upon the bone of the rat Reprinted by permission from Selye H and Horava A *Second Annual Report on Stress* Montreal ACTA Inc 1952

Having thus connected STH with adrenal function it may be of interest to discuss another experiment although it is not directly concerned with the plurality of adrenal hormones Figure 5 illustrates the selective conditioning of STH by a folliculoid compound stilbestrol I think it is quite proper to speak here of selective conditioning since not all the actions of STH are influenced by folliculoids and vice versa Figure 5 shows sagittal cross sections through the lower extremity of the femur in three rats On the left is the normal bone structure in an untreated control animal In the middle there is a dense sclerotic femur in which much of the marrow has been replaced by trabecules of spongy bone This animal was simultaneously treated with STH and stilbestrol On the right there is a bone with very little sclerosis after treatment with stilbestrol alone STH alone (not illustrated here) enlarges all parts of the femur proportionately without causing any sclerosis

It was concluded from this experiment that although in the rat folliculoids cause very little bone sclerosis and STH causes none combined treatment with the two hormones results in a selective conditioning for this particular effect of the growth hormone so that the latter induces pronounced sclerosis of certain parts of the skeleton (9) Through what intimate mechanism of action the growth hormone is so modified in its effect upon the bone as to cause this sclerosis I would

Histologic examination showed that in most of the rats treated with both pituitary hormones there developed extensive necroses in the adrenals similar to those which were previously obtained with LAP (5) a preparation which contains both STH and ACTH Indeed, in one of these animals an animal which died with extraordinarily large suprarenals there was actually no living adrenal tissue at all since histologically both adrenals had been replaced by necrotic tissue This type of observation raises the possibility of a fatal adrenal insufficiency actually being induced by excessive corticotropic stimulation Perhaps these necroses could be avoided if somewhat smaller doses of the pituitary hormones were given and the enlargement developed more gradually Such experiments as well as studies on the effect of combined ACTH and STH treatment in hypophysectomized rats, are now being conducted by my associate Dr Salgado

Another interesting side observation made in the course of these experiments was that the so called brown fat which always surrounds the adrenal of the rat was very much stimulated by the combined treatment with STH and ACTH These adrenals were suspended in a gelatinous mass of connective tissue containing brown fat cells which had assumed an appearance strikingly similar to that of stimulated adrenal cortical cells

Li How about the growth hormone alone?

Selye Growth hormone alone also caused marked adrenal enlargement but only at dose levels which produced a considerable increase in somatic growth rate Since this effect was not seen in hypophysectomized animals I am tempted to ascribe it either to the liberation of ACTH by the animal s own pituitary under the influence of STH or to some extrahypophyseal synergism between STH and endogenous corticotrophin

Li How about the histology?

Selye Growth hormone alone, at the dose levels tested so far causes a fairly normal enlargement of the adrenal cortex that is one un accompanied by necrosis excessive hyperemia or excessive lipid loss I might add that with one of Armour s growth hormone preparations which was virtually free from ACTH as judged by its effect upon the adrenals of hypophysectomized animals I have obtained adrenal weights up to 200 to 250 mg in rats In one case the adrenals weighed 394 mg It must be said that this was seen only after several months of treatment the daily dose of STH being raised in proportion to the increased body weight

Li Is the adrenal cortex larger in terms of 100 grams of body weight?

Selye Oh yes I shall come back to this in connection with the conditioning of hormone effects

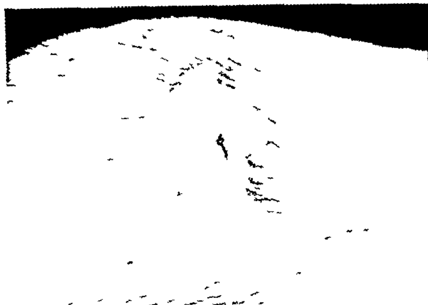


FIGURE 7 Closer view of the hydrocortisone deposit in an experiment similar to that illustrated by Figure 6 Reprinted by permission from Selye H and Horava A *Second Annual Report on Stress* Montreal ACTA Inc 1952

months before this picture was taken Although histologically the skin structure remained essentially normal over the air space hair growth was delayed presumably due to some trophic disturbances The hydro cortisone (1 mg of microcrystals) was deposited into the subcutaneous connective tissue the day before air insufflation yet the size of the deposit hardly diminished in the following two months Dilated veins are particularly visible in the atrophic skin area which covers and sur rounds the crystals Here all layers of the skin are extremely atrophic

Figure 7 is a closer view of the hydrocortisone deposit in a similar experiment Note particularly the complete inhibition of hair growth in the atrophic skin area which surrounds the hormone accumulation It is noteworthy in connection with the problem of conditioning that a similar deposit of hydrocortisone acetate placed in the loose subcutane ous tissue of the same rat outside the pneumoderma has little if any effect on the skin except on the very limited area which immediately covers the crystals Apparently the cutaneous dystrophy caused by the pneumodermal procedure so sensitized the skin to the action of this gluco corticoid that the limited hormone deposit was capable of affect ing a comparatively large area of the surrounding skin Qualitatively

not venture to say It is my impression however on the basis of histologic studies that folliculoids inhibit osteoclastic bone resorption while STH stimulates both bone formation and bone resorption When both hormones are simultaneously given the latter effect of STH is blocked by stilbestrol, while its ability to stimulate osteogenesis persists The result is a change which greatly resembles the experimental marble bone disease, as we produced it with parathyroid hormone many years ago I should like to show some work which yielded techniques found to be especially informative in the study of certain corticoid actions By the subcutaneous injection of 60 to 100 ml of air in the interscapular region of the rat it was possible to separate the dorsal skin from the underlying muscle and fascial layers The skin thus detached remained perfectly viable but underwent a type of dystrophy *which was selectively altered in response to various topically applied* agents among others to corticoids In analogy with the designations pneumoperitoneum or pneumothorax this might be refered to as a pneumoderma

Figure 6 shows a pneumoderma with a hydrocortisone acetate deposit Note the limited air sac on the back of this rat and the white hormone deposit The hair was removed from the back with barium sulfide two



FIGURE 6 Pneumoderma with a hydrocortisone deposit Reprinted by permission from Selye H and Horava A *Second Annual Report on Stress* Montreal ACTA Inc 1952

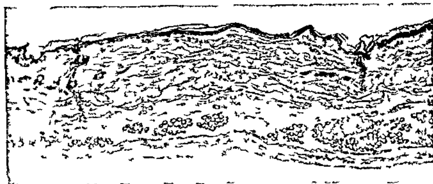


FIGURE 9 Microscopic appearance of the skin detached by the pneumoderma technique This section was taken from the immediate vicinity of the hydro cortisone deposit

especially this region which we examined by palpation at frequent inter vals during the experiment Particularly noteworthy is the fact that even the striated musculature underwent intense atrophy in the vicinity of the hormone deposit Such muscle atrophy was not produced by the pneumoderma itself nor by a similar hydrocortisone deposit injected into ordinary that is not detached skin Apparently under these con ditions hydrocortisone caused atrophy of striated musculature by a direct topical effect

Pincus Is this the acetate or the free alcohol?

Selye The acetate The surprising thing was that although the skin became extremely thin it never perforated Apparently despite its thin ness it was highly resistant

It seemed to us that this technique might be quite interesting for the bioassay of extremely small amounts of antiphlogistic or gluco corti coids If the skin is merely insufflated and microcrystals deposited a very small amount of hormone can be tested for its ability to cause atrophy of connective tissue even in the absence of any inflammatory phenomenon For bioassay purposes we take a sheet of cardboard and after excising the skin detached by the pneumodermal technique we fix it to the cardboard with a stapler in a flat position Then the card board with the skin is immersed in formalin or some other fixative After fixation the flat sheet of fixed skin which having lost its elastic ity maintains its shape is punctured several times with a paper puncher in the area immediately surrounding the hydrocortisone deposit as well as in the immediately surrounding but still detached area which was not influenced by the hormone The small disks which have been thus punched out can be weighed exactly on an analytical balance The mean

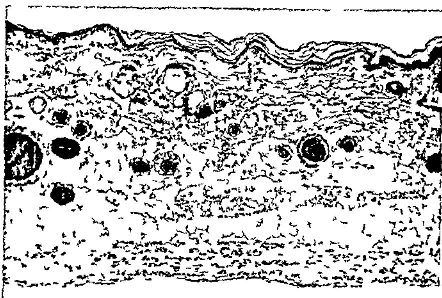


FIGURE 8 Microscopic appearance of the skin detached by the pneumoderma technique This section has been taken at a distance from the hydrocortisone deposit

the actions of hydrocortisone so applied were essentially similar to those described by Drs Baker and Ingle after direct application of adrenocortical extract or of purified gluco corticoids to the skin in alcoholic solution However such topically administered corticoid solutions like the subcutaneous injections of hydrocortisone under normal skin exert their effects only at the site of application without the peculiar phenomenon of the spreading atrophy seen in these pictures

Figure 8 shows the essentially normal structure of the detached skin in the pneumodermal region when taken from a region far away from the hormone deposit There was some atrophy of the subcutaneous fat tissue and the hair follicles were perhaps not quite as numerous and well developed as they would be normally Otherwise the skin retained its usual appearance The slight inflammatory changes (some round cell infiltration particularly underneath the muscular layer of the skin) were probably due to repeated palpation of this region They were not noted in many other experiments in which special care was taken not to injure the derma mechanically

Figure 9 shows a similar skin section taken from the same animal but from the atrophic skin area immediately surrounding the hydro cortisone deposit Here there were no hair follicles or sebaceous glands Inflammatory changes were completely suppressed although it was

posit This in itself shows that the amount absorbed and responsible for the extreme atrophy in the surrounding skin area must have been very little

Ralli Dr Selye is this a drawing of the microphotograph and what staining has been used?

Selye That is the microphotograph itself Hematoxylin eosin stain was used It looks like a drawing because the photograph was taken with a very narrow diaphragm which brings out the contours sharply

Sayers Wouldn t it be possible to extract the tissue and analyze for 17 hydroxycorticosterone?

Selye It would

Sayers Have you done studies of that type?

Selye No we have not

I should like to show a modification of this technique which we call the granuloma pouch If a smaller amount of air is injected let us say 25 ml into the loose subcutaneous tissue of the interscapular region a circumscribed air bubble is formed This can be transformed into a veritable granuloma pouch by the subsequent injection into the lumen of some irritant such as kaolin formalin or croton oil Usually we inject 1 ml of peanut oil or corn oil containing 1 per cent croton oil This causes pronounced irritation and transforms the loose connective tissue which surrounds the air bubble into a thick, inflamed membrane This membrane becomes well delimited from the surrounding tissue and the air in its cavity is gradually replaced within 14 days by a fluid exudate

We feel that the granuloma pouch technique has an advantage over the usual procedures for the study of experimental inflammation in that it permits us to produce consistently an accurately measurable granu loma mass and exudate Furthermore it facilitates the objective and accurate estimation of absorption from the inflamed surface into the blood or lymph stream as well as the estimation of the excretion of blood borne products through the inflamed barrier We have used the procedure particularly for the objective assessment of the functional value of granuloma tissue for instance its resistance to the topical effects of microbes chemical and mechanical irritants etc

In connection with the antiphlogistic effects of corticoids it is espe cially noteworthy that if hydrocortisone is injected subcutaneously as in the previously mentioned pneumodermal experiment and the loose connective tissue containing the hydrocortisone deposit is detached from the underlying tissues with 25 ml of air the subsequent injection of an irritant into this bubble no longer produces any definite inflammatory changes

difference between the weights of the disks coming from the thin area and from the surrounding skin respectively is taken as an objective indicator of the degree of cutaneous atrophy thus produced To give an example the average weight of such disks in this experiment was 32 4 mg (with a standard error of 2 1) in the detached skin, just outside of the region directly influenced by hydrocortisone, and 11 1 mg (with a standard error of 0 9) in the atrophic areola surrounding the hormone I might mention incidentally that if some inflammatory infiltration occurs in the detached skin as a result of handling it is very often predominantly eosinophilic

Sayers This is without hydrocortisone?

Selye Without hydrocortisone No actually I should say it is with hydrocortisone but in a region of the skin which is at a great distance from the hydrocortisone deposit

White Have you done this serially so that you know eosinophils are the cells which predominantly come in all along after the injection of the area?

Selye No

White These illustrations are from animals studied how long after the injection of the area?

Selye This particular one (Figure 9) is at two months I haven t followed the response longer

I mentioned these experiments in connection with the mechanism of action of corticoids because it has always impressed me as a definite gap in our investigations that no one has as yet adequately studied the influence of hormone fixation in tissues It should be emphasized that quite apart from dystrophic changes in the pneumoderma absorption from it is greatly delayed For instance a hydrocortisone deposit which persists several months in the connective tissue of the pneumoderma would not persist for more than a few weeks if it were injected into normal subcutaneous tissue without air insufflation Obviously the procedure fixes the hormone deposit at the site of the injection perhaps as a result of a locally impaired blood supply The question arises whether differential fixation of hormones can occur naturally in various tissues of the same organism If so selective effects upon individual targets would be explained even if the blood level or urinary excretion of the hormone remained unchanged

It is obvious that if such fixation is artificially induced by the pneumodermal technique the hormone becomes extremely effective However we have no proof that this is exclusively due to fixation Presumably the nutritional disturbances in the skin also act as conditioning factors At any rate in the whole course of this prolonged experiment we were unable to detect any visible diminution in the size of the de

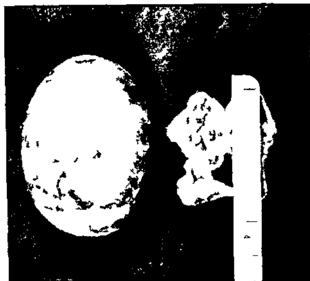


FIGURE 11 Macroscopic appearance of a fresh granuloma pouch in an untreated rat (left) and one in which inflammation was inhibited due to the injection of hydrocortisone into the granuloma wall (right)

dissect it as a complete spheroid membrane I inserted a tape measure into the lumen of the membrane I did manage to dissect to show that it is so thin that one can easily see through it Actually this is almost completely unaltered ordinary connective tissue lined by epithelioid cells

To me the surprising thing is that in ordinary skin the topical effect of hydrocortisone is limited to the immediately adjacent area in the dystrophic skin of the pneumoderma this effect spreads considerably further and in the granuloma pouch the spreading is even greater The hormone was deposited in a very limited area at one point of the pouch and it influenced all the connective tissue surrounding the air bubble even that situated several inches away at the pole opposite to the place where the hormone was deposited At the same time exudate formation was virtually absent even after 14 days the cavity contained only air and the irritant which was originally injected

Manifestly under these particular circumstances the hydrocortisone managed to condition the influence of a very large surface of connective tissue Yet its effect was still topical since injection of the same amount of hormone into normal subcutaneous tissue at only a few millimeters from the pouch wall remained completely without effect permitting the development of a thick inflamed granuloma with a voluminous exudate



FIGURE 10 Macroscopic appearance of a granuloma pouch after fixation and evacuation of the contents

As can be seen from Figure 10 the granuloma pouch produced with 25 ml of air plus 1 ml of 1% croton oil can easily be enucleated by *simple pressure after splitting the skin surface* The pouch was fixed *in toto* and opened by a partial transverse section to show the regularity of both the outer and the inner surface

Figure 11 shows on the left a similar granuloma pouch without cortisone treatment Here can be seen the exudate level as distinct from the lighter area occupied by the residue of air because the tissue is not fixed it is not seen distinctly because of the thickness of the pouch wall *On the right is the corresponding structure from a rat* treated exactly the same way as that whose pouch is shown on the left but here one milligram of hydrocortisone was deposited into the loose connective tissue surrounding the air bubble This structure—it can hardly be called a pouch—is extremely thin and therefore I could not

Pincus In the hydrocortisone area is there an inhibition of hair growth?

Selye Oh yes In the hydrocortisone areola there is never any trace of hair growth whether a pneumoderma is produced or a granuloma pouch This however only confirms what Drs Ingle and Baker have shown by topical painting of the skin with solutions of corticoids

Long Have you any idea what the air pressure is within that sac particularly in relation to the venous pressure?

Selye We have never actually measured it

Long Offhand one would think that the vein would collapse when the air pressure gets higher than the venous pressure

Selye When the air is injected there is very little pressure immediately afterwards The pouch is soft It is easily compressed between two fingers However if the pouch is irritated for instance by the injection of croton oil as shown in Figure 11 then the pressure becomes very high This is partly due to exudate formation and partly to the secondary contraction of the granuloma pouch which occurs after much fibrous tissue develops in its wall In those animals in which the pouch was irritated with croton oil but protected by hydrocortisone the outstanding histologic feature was the virtually complete absence of connective tissue fiber formation and hence of contraction The extremely thin wall in these cases consisted almost exclusively of roundish or polyhedral epithelioid cells Since this structure was incompatible with a cicatricial contracture the hydrocortisone protected pouches were always extremely flabby and of course devoid of exudate If the pouch was filled only with air and was not otherwise irritated then it remained quite flat and the pressure in it was never very high

Conn Are you going to try DCA and compound F mixed together and injected into the same area?

Selye I have done that I did not plan to report on it as yet because the DCA is absorbed too rapidly and I am not quite sure about the interpretation of my results The experiment was done with the ordinary pneumoderma not with the granuloma pouch technique and DCA and hydrocortisone were injected in the area simultaneously after being mixed in the same syringe The skin atrophy was just as marked as if no DCA had been injected

I forgot to mention that various irritants produce qualitatively different types of granuloma tissue Thus formalin tends to cause necrosis or at lower dose levels predominantly fibroblastic reactions croton oil a predominantly hemorrhagic exudate with an intensely fibrous capsule mustard powder results in the production of innumerable foreign body giant cells turpentine elicits predominantly pus formation We are now trying to examine the effects of various hormones on these

Pincus When formalin is used as the irritant is it injected into the air cavity the pneumoderma?

Selye Yes it is injected into the cavity immediately after the air is and then the animal is shaken up in order to distribute the irritant equally on the surface of the cavity

Sayers The irritant does not fill the cavity?

Selye No

Li Have you injected growth hormone into the pouch?

Selye I have but it is very rapidly absorbed and nothing happens Perhaps one should administer it simultaneously with substances which delay absorption If growth hormone is repeatedly injected outside the pouch, just subcutaneously in the same animal there is a marked skin hypertrophy which is not limited to the area of injection as Drs Ingle and Baker have shown

Pincus But if it is injected locally?

Selye Then a single injection is given which is very rapidly absorbed You see it isn t only that absorption is delayed in the detached skin but that hydrocortisone acetate is very slowly absorbed anyway If hydro cortisone acetate is put into the wall of the pouch the absorption rate is as slow as can be A water soluble substance like growth hormone is too readily absorbed for such single injection experiments If a growth hormone tannate could be made as there is a pitressin tannate or some other slowly absorbable preparation of STH then one might be able to use it As it is it cannot be employed for this kind of work

Thorn Is the circulation in the pouch too slow to permit the gas content of the pouch to be used for respiratory studies?

Selye Comparing arteriovenous values?

Thorn I was thinking of over all respiratory quotient studies

Selye We have made no chemical measurements on the pouch air If one compensates for the absorption of the air by injecting additional quantities a pouch can be maintained for months

Thorn I was thinking that you might be able to use the air in the pouch for the purpose of making respiratory quotient studies following the feeding of food and so forth which would bypass the technical difficulties of mass respiratory quotient studies in animals

Selye I think the oxygen is absorbed from the pouch very rapidly while the nitrogen stays behind In any case we have not done any study of this kind but I may say that although the detached part of the skin is poorly vascularized the base of the pouch is very rich in vessels

Vogt What happens to the hair Dr Selye?

Selye We usually pluck the hair or remove it with barium sulfide But it can grow back even in the area of the pneumoderma although it is greatly delayed there

Chow) *ad lib* After 15 days when this experiment was terminated their body weight was 136 Gm and their adrenal weight 35 (± 1 7) milligrams Group II like all the other groups was pair fed with group III that is they received just as much food as was required to maintain their body weight approximately equal to that of group III Since group III received cortisone and grew very little this meant a considerable artificial reduction in the food intake of group II At the end of the experiment the mean body weight in this group was 112 Gm and the adrenal weight 27 (± 1 2) milligrams

Group III was treated daily subcutaneously with 2 5 mg of cortisone acetate Here the final body weight was also 112 Gm The adrenals underwent the usual compensatory atrophy weighing 16 (± 1 1) milligrams These rats received food *ad lib* but grew very slowly because of the cortisone overdosage

Group IV received the same amount of cortisone as group III but they also were simultaneously treated with 4 mg of Armour s STH daily subcutaneously Their food intake was likewise so reduced by pair feeding that their growth curve was essentially similar to that of the rats in group III Their body weight was 116 Gm and their adrenals weighed 26 (± 1 6) mg at the end of the experiment

Group V received STH alone at the same dose level as group IV Here again the food intake was restricted so that their body weight remained essentially at the same level as that of the rats in groups III and IV At the end of the experiment their mean body weight was 112 Gm and their adrenals weighed 41 (± 2 1) milligrams

These figures I think conclusively prove that the corticotropic effect of STH is not merely a secondary consequence of its growth promoting action I use the term corticotropic not in the sense of a direct effect upon the adrenal cortex although this is not excluded but merely to denote adrenocortical stimulation whether mediated through the pituitary or not In any event the adrenal cortex enlarged under the influence of STH even if the growth promoting effect of this hormone was artificially prevented by reducing the food intake Under the same conditions that is by equalizing the body weight by restricted feeding STH also antagonized the compensatory adrenocortical atrophy normally induced by cortisone Let me emphasize that this particular STH preparation had virtually no corticotropic effect in hypophysectomized animals Hence I think it can be said as a general conclusion that its effect was not due to contamination with ACTH and that whatever the mechanism through which STH enlarged the adrenal of the intact animal it was not merely because it acted as a growth hormone

Long There was no growth promotion difference with STH between II and V?

different types of response to topical irritation using the granuloma pouch technique

Pouches can also be produced by microbes or you can purposely infect a pouch which is or is not under the influence of hormone therapy study and thus the functional effect of hormones on the spread of an infection I need not go into all the obvious endocrinologic implications of this We have also tested the effect of systemic treatment, i e, injections at a distance from the pouch of cortisone ACTH and STH In these experiments the hormones were injected daily during the entire development of the pouch As expected, cortisone and ACTH make the granuloma thin while STH causes it to proliferate and results in very thick walled granuloma pouches We are in the process of using the same technique for the study of various other problems, for instance the effect of systemic stress upon granuloma and exudate formation

Ralli What size needle is used to form or inject the pouch?

Selye We use a No 25 hypodermic injection needle for the first insufflation of the pouch and the introduction of the irritant However after the granuloma is well developed it can be punctured with a heavier needle to remove specimens of the sometimes rather thick exudate which may not go through the No 25

I should like to present a few data on the interactions between cortisone and STH In earlier studies we had noticed that if normally lethal doses of cortisone were given at the same time that STH was injected in quantities adequate to maintain a normal body weight then the lethal effects of the gluco corticoid were abolished or at least mortality was diminished It will be recalled that Dr Evans and Dr Li showed some time ago that the effect of ACTH on the junction cartilages is opposed by STH We found that the opposing effects of these two types of hormones upon inflammation the spreading of infections and many other phenomena were also mutually inhibited in the case of simultaneous treatment with both types

It is interesting that even the adrenocortical atrophy normally produced by cortisone can be abolished by simultaneous treatment with STH This was rather unexpected and we wondered whether here STH acts upon the adrenals only secondarily as a consequence of metabolic changes connected with its growth promoting effect We therefore attempted to eliminate the growth effect of STH to see whether it would still stimulate the adrenal cortex In a very recent experiment we found the following There were five groups each of eight female rats in this experiment and these were so selected that in each group the average body weight was 102 Gm at the beginning Group I consisted of untreated normal animals which received food (Purina Fox

STH become unusually large even if the animals receive food *ad lib* and grow at an excessive rate As a matter of fact it is under these conditions that we first noticed this corticotropic effect of STH

Li In proportion to body weight though

Selye Oh no with high doses of STH the adrenals grow far more than in proportion to body weight

Ingle Dr Selye have you studied the effect of growth hormone on adrenal size in the hypophysectomized rat given a uniform intake of corticotrophin in amounts just sufficient to prevent adrenal cortical atrophy?

Selye No but that is an experiment which Dr Salgado is doing now I think we shall get definite results on this in a few weeks One possibility is that the corticotropic effect of STH would manifest itself only if the adrenal was maintained in a responsive condition by STH treatment

Ingle Was this Armour s growth hormone?

Selye Yes

Sayers Dr Selye could you not explain these data by a contamination of your STH with ACTH?

Selye No because the same STH preparation when given in the same amount to hypophysectomized animals never gave any corticotropic effect in our hands

Sayers You mean it is completely free from ACTH?

Selye Armour s tells us it contains a trace but we could not detect it by using merely adrenal weight as an indicator In any event the ACTH contamination in our STH must be so small that it could not account for the pronounced adrenal enlargement which I repeat we obtained only in intact animals Maybe Dr Li knows how much it contains I have forgotten the exact figure in units but it contains very very little

Li A very very small amount

Selye It contains also a thyrotropic hormone

Sayers I should like to know just how much it contains in order to evaluate an experiment of this kind

Lukens I think that the figure for Armour s growth hormone is about one tenth of one per cent of ACTH on a milligram basis

Pincus How much STH was given per day?

Selye That was 4 milligrams

Sayers In two doses?

Selye In two doses

Li The content of STH is very small

Selye We went up to 5 mg of the same preparation in hypophysectomized animals and obtained virtually no adrenal hypertrophy

Selye They were purposely so fed as to have the same weight i e group V received much less food so that they would have the same weight as the animals in groups II III and IV That is what I meant by paired feeding in this case The normal untreated rats in group I and the merely cortisone treated animals of group III were fed *ad lib* All the others were fed in such a manner that more food was given when they became lighter than and less food when they became heavier than the merely cortisone treated animals of group III

Thorn That is not pair feeding though

Selye The food was arranged so as to arrive at the same final body weight Pair feeding is perhaps not the right term

Pincus Restricted feeding

Selye How could we express the fact that their food intake was not merely restricted in an arbitrary manner but that they were fed so as to end up with the same body weight?

Pincus Controlled feeding

Selye It should be corrected then and I should have spoken of controlled feeding

Ralli Do you happen to have the thymus weights on these animals?

Selye I do not have the figures with me because this experiment was terminated only a few days ago We fixed the thymus glands for weighing as were the adrenals but up to now we have weighed the adrenals only

Pincus Why do you conclude that this is a growth effect and not a nonspecific stress effect of the STH preparation?

Li The food intake of the group V animals must have been tremendously smaller than that of group II so much smaller that they ended with starvation actually What was the actual food intake each day in group V?

Selye We didn t measure it in this experiment but we are going to measure it in a similar one which we started after completing this and we shall publish our results in detail later I may say however that the growth hormone treated animals required less food to maintain the same body weight Would you have expected this?

Li A growth hormone injected animal would eat more So in order to restrict the diet for this animal to grow to the same extent as the untreated animal it would require much less food

Selye It was our impression that they required much less food to stay at the same weight Have you done any such experiments?

Li Yes we have had some experience with that

Selye It is of course possible as Dr Pincus said a minute ago that giving STH on a restricted diet which does not permit growth creates a situation of stress However the adrenals of rats overdosed with

the body weight is relatively well maintained under these circumstances

Li It is still a stress because the animal requires more food in order to grow

Thorn If the caloric intake of a patient with myxedema is reduced this does not call forth the same reaction in the body as when the caloric intake is restricted to the same level in an individual whose metabolic rate is normal

Selye Pomeranz showed that the greatest adrenal hypertrophy occurs on complete acute starvation while chronic maintenance on a reduced food intake causes adrenal atrophy If food is completely withheld there is marked protein catabolism and the adrenal becomes very large This is presumably a manifestation of stress In other words it might be stated this way growth hormone causes an adrenocortical hyper trophy whether growth is permitted by giving food *ad lib* or restricted by maintaining the animal on an insufficient food intake If you want to call this reaction a stress effect I agree but then we would have to say that whatever metabolic changes growth hormone effects whether causing growth or not it always is a stressor for the body and it always causes adrenal growth By this time I think the whole matter becomes a question of semantics

Pincus It is not a question of semantics if you assume that this stress involves ACTH release You are not talking about definitions you are talking about the production of something that stimulates the adrenal

Selye I think STH does cause ACTH release since in the hypophy sectomized animal it does not produce adrenal hypertrophy and in the intact animal no matter what happens to growth it does cause adrenal hypertrophy I think the most plausible explanation is that STH has as one of its specific effects the ability to induce a considerable ACTH discharge from the hypophysis However the manifestations of this ACTH discharge are largely blocked because of the antagonism between STH and ACTH as regards their effect upon most organs In our very early experiments with LAP we were struck by the production of a singular set of symptoms namely very much enlarged adrenals gen eralized inflammatory changes myocarditis arteritis arthritis a large thymus and a large spleen This combination of changes could not be ascribed either to ACTH or to STH It was difficult to explain and we could only say that it was the result of treatment with an impure pitui tary preparation Now however we assume that it was due largely to STH which is plentiful in LAP and to the endogenous ACTH re leased under the influence of the former

Pincus We have perfused adrenals with LAP and found no measur able effect on steroid release This agrees with the direct effect of LAP of which you speak

Sayers If one unit is taken as equivalent to one milligram of the standard preparation the rats received 1 milli units of ACTH per day This is not an insignificant quantity of ACTH

Astwood That would have no effect on adrenal weight would it?

Selye Especially *if given in only two injections*

Pincus It would have a marked effect on the ascorbic acid depletion

Sayers Five milli units injected once per day have an appreciable effect on adrenal weight in the hypophysectomized rat (10)

Selye Fourteen milligrams of adrenal weight were added between groups II and V although the final body weight was the same If a larger dose namely 5 mg of the same STH is given to hypophysectomized rats under the same circumstances by two injections a day in water as we have done it here there is no appreciable increase in adrenal weight I do not doubt that it might deplete ascorbic acid

Sayers The administration of cortisone reduces adrenal weight and in a sense produces the same situation as in a hypophysectomized rat We know that the hypophysectomized animal is much more sensitive than an intact animal to small doses of ACTH as far as adrenal weight is concerned The administration of cortisone is a sort of chemical hypophysectomy

Selye If you consider it as such then the difference between group III and IV should be duplicated on similar animals after hypophysectomy and that we didn t find to be the case

Pincus May I ask *for an answer to the question I proposed originally* Why don t you interpret this as a nonspecific hypertrophy of the adrenal rather than as a specific growth hormone effect in group V?

Sayers The effect is probably the result of endogenous release of ACTH

Pincus Yes

Selye In the animal which is fasted it may be argued that the animal is under greater stress if growth hormone is simultaneously given than if it is fasted without the growth hormone This case could be interpreted that way But I am not very much inclined to ascribe the whole STH effect to stress under all conditions because as I said before even if free growth is permitted and the animal is obviously in fine condition there is still the corticotropic effect

White The question could be answered if there were one more group of animals in group I restricted to the same food intake as is group V

Thorn If growth hormone prevents weight loss despite a reduction in food intake it really is not an added stress is it? In other words these animals are not starved more than control animals in the group The stress of not eating must be considerably less on the organism if

the body weight is relatively well maintained under these circumstances

Li It is still a stress because the animal requires more food in order to grow

Thorn If the caloric intake of a patient with myxedema is reduced this does not call forth the same reaction in the body as when the caloric intake is restricted to the same level in an individual whose metabolic rate is normal

Selye Pomeranz showed that the greatest adrenal hypertrophy occurs on complete acute starvation while chronic maintenance on a reduced food intake causes adrenal atrophy If food is completely withheld there is marked protein catabolism and the adrenal becomes very large This is presumably a manifestation of stress In other words it might be stated this way growth hormone causes an adrenocortical hypertrophy whether growth is permitted by giving food *ad lib* or restricted by maintaining the animal on an insufficient food intake If you want to call this reaction a stress effect I agree but then we would have to say that whatever metabolic changes growth hormone effects whether causing growth or not it always is a stressor for the body and it always causes adrenal growth By this time I think the whole matter becomes a question of semantics

Pincus It is not a question of semantics if you assume that this stress involves ACTH release You are not talking about definitions you are talking about the production of something that stimulates the adrenal

Selye I think STH does cause ACTH release since in the hypophysectomized animal it does not produce adrenal hypertrophy and in the intact animal no matter what happens to growth it does cause adrenal hypertrophy I think the most plausible explanation is that STH has as one of its specific effects the ability to induce a considerable ACTH discharge from the hypophysis However the manifestations of this ACTH discharge are largely blocked because of the antagonism between STH and ACTH as regards their effect upon most organs In our very early experiments with LAP we were struck by the production of a singular set of symptoms namely very much enlarged adrenals generalized inflammatory changes myocarditis arteritis arthritis a large thymus and a large spleen This combination of changes could not be ascribed either to ACTH or to STH It was difficult to explain and we could only say that it was the result of treatment with an impure pituitary preparation Now however we assume that it was due largely to STH which is plentiful in LAP and to the endogenous ACTH released under the influence of the former

Pincus We have perfused adrenals with LAP and found no measurable effect on steroid release This agrees with the direct effect of LAP of which you speak

I should like to ask what histologic effect there is on the group V adrenals when they are compared with the others? If an ACTH effect is present, there might be indications from such things as lipid depletion

Selye They are rich in lipid

Li How about the medulla?

Selye We have never seen any change in the medulla That is another thing which is worth mentioning The figures which you have published (11) are perfectly convincing to me but we never obtained such changes

Li If the hormone is injected for a period of over a year say fourteen months in rats the weight of the adrenals is about double their original weight But as for the histology only the medulla is hypertrophied not the cortex

Lukens Dr Selye would it clarify this situation and answer Dr Styers question if these experiments were conducted with 1 mg of growth hormone a day? This is known to be an amply effective dose for growth and nitrogen retention in the rat This dose would contain so little ACTH if our experiments are correct that it would answer the objection of contamination with ACTH Has that been done? Have you tested 1 or 5 mg of growth hormone for its ACTH content in hypophysectomized rats?

Selye We gave up to 2 5 mg twice daily in aqueous solution to hypophysectomized animals without obtaining any significant increase in adrenal weight We did not determine the ascorbic acid of these adrenals However under exactly similar conditions in the intact animals the adrenals become very large as a result of the same STH treatment If ACTH is given without any retarding agent in aqueous solution twice daily it is singularly ineffective

Long I was very much interested in Dr Selye s statement as to the inhibition of the effects of cortisone by growth hormone but I believe I know one situation where they apparently do not inhibit but rather *synergize each other and that is in the production of glycosuria* It has been shown I think by three different laboratories that in the rat the dog and the cat where either hormone alone is relatively ineffective a combination of the two will produce quite a marked glycosuria Perhaps in the same vein one might say that as Dr Selye mentioned in the absence of the hypophysis these DCA effects are greatly reduced Certainly the effects of cortisone on carbohydrate and protein metabolism appear to be just as marked in the hypophysectomized animal as they are in the normal animal

Selye We have done blood sugars on animals chronically treated with cortisone and STH and as far as I can remember we have never

had a blood sugar over 180 under our experimental conditions in the rat Yet in general the blood sugar of animals simultaneously treated with STH and cortisone tended to be higher than that of controls receiving cortisone alone Of course I am far from believing that all the effects of STH are antagonized by cortisone and vice versa I am well aware of the reported synergism as regards carbohydrate metabolism but there may be species differences and the rat is particularly resistant to the production of diabetes by this combination of hormones Even differences in the strain of rats make for great variations as regard diabetes in the rat

White Does someone recall better than I Dr de Bodo s report at the Laurentian Hormone Conference? I had the impression that he said the diabetogenic effect of growth hormone in the hypophysectomized dog was counteracted by cortisone

Selye Yes that is correct

Lukens That is correct except that I think he was measuring the action of insulin rather than the production of glycosuria

Li There are other situations where these two hormones act synergistically One such situation is the appearance of ketonemia and ketonuria in fasted normal rats As we have reported (12) both growth hormone and ACTH have ketogenic activity Even in the absence of the adrenal the ketogenic effect of growth hormone is apparent Another example is the increase of liver fat content in hypophysectomized and normal rats through the effects of these two hormones (13) I should like to comment on this antagonistic effect between cortisone and growth hormone For a number of years we have been injecting growth hormone into both normal and hypophysectomized rats for periods of over a year s duration We have found that in the normal animal tumors are produced with growth hormone whereas none are formed in hypophysectomized animals We have been thinking recently that perhaps the adrenal or the gonads might be mediating factors in this phenomenon of the formation of tumor after long term injection of growth hormone

Selye These are lymphatic tumors?

Li Yes but not only lymphatic There have also been tumors present in the medulla for instance

For the past year or more Dr W O Reinhardt and I have been injecting growth hormone into a group of castrated adrenalectomized rats whose pituitaries were still intact After the first three months of injection we noticed that the animals had developed a condition with all the symptoms of chronic arthritis the joints were swollen and their locomotion was greatly impaired Radioautographic studies of the bone showed a typical arthritic condition These symptoms were present in all of the adrenalectomized castrated animals which had been treated

with growth hormone in only one of the normal growth hormone treated controls and in none of the untreated controls We took two of the adrenalectomized castrated animals which were in poor physical condition and injected them with 1 mg of hydrocortisone Within two or three days this treatment seemed to relieve the symptoms The growth rate of these animals continued to be the same as that of normal animals with intact adrenals This experiment seems to show that growth hormone does participate to a certain extent in arthritic conditions in the rat and that hydrocortisone in some way counteracts this effect of growth hormone

Selye What type of arthritis is it?

Li I cannot say since the animals are still being injected with growth hormone and the tissues have not yet been studied The arthritic results were only incidental the primary object of the experiment being the production of tumor in adrenalectomized-castrated animals However it is interesting to note that in not a single one of the normal animals treated with growth hormone has there been an arthritic condition produced this condition occurred only in the absence of the adrenals or when they were atrophied as a result of hypophysectomy It would appear that the growth hormone had no alarm effect on the animal

Corn How were these animals maintained?

Li With saline

Ralli Did you say you had to castrate as well as hypophysectomize the animals in order to produce the arthritis?

Li We don t know These are as I said incidental observations The experiments were performed because we had thought that tumor formation might be due to the production of a gonadotropic substance or to the production of a certain form of ACTH by the pituitary

White The reason I asked previously about the ketogenic effect is that I rather like the idea of a balance between growth hormone and ACTH in the sense that a balance of factors fits in with the general picture of some part of a homeostatic mechanism I recall that we ourselves and other people have described for example a marked effect of cortisone or adrenocortical extract on lipid mobilization One begins to wonder how many of those experiments must be repeated in the hypophysectomized animal in order to determine whether those data are attributable to a secondary response of endogenous growth hormone in an attempt to counteract the effect of cortisone

The capacity of the human pituitary to produce ACTH might account in part for the fact that growth hormone has to the present time produced very little effect on man Could it be that there is a degree of ACTH production in response to growth hormone injection in man which results to some degree in a negation in this species of any effect

that might be expected of growth hormone?

Ralli It might be a matter of the dose

White Yes in any balance of this type there is generally a quantitative relationship I don t know that anyone would want to try or has tried using heroic doses of growth hormone in man I don t know if it would be particularly advisable But one does wonder whether there is a dose level at which man may respond to growth hormone I think that the first doses of cortisone which were used by the Mayo group in rheumatoid arthritis were considered at the time to be rather heroic doses and perhaps the same situation obtains with respect to growth hormone

Ralli A situation that gives a clue to dosage may be the overgrowth of tissue that occurs in the patient with acromegaly which is due to an eosinophilic tumor of the pituitary I do not know of any recent assays for the growth hormone activity of these tumors nor do I know what other pituitary hormones might be increased in this situation It would appear that the growth hormone is decreasingly effective as maturity is approached but in the acromegalic who is a mature individual an overgrowth of bone and of other organs is produced i e the liver the kidneys the spleen

Selye In connection with Dr White s remark concerning the insensitivity of the human being to STH it is perhaps of interest that based on a very similar theoretical consideration we have given STH plus DCA on the assumption that DCA does not have any catabolic effect but does cause compensatory adrenal atrophy and might to some extent compensate for any ACTH secretion that the STH would induce We found in fact that small doses of DCA given to nonsensitized animals increase the growth effect of threshold doses of STH in the rat

A second relevant fact is that in recent experiments performed in collaboration with Drs Mitchell and Guillemin we found that the guinea pig is entirely insensitive to the growth effect of such STH preparations as are now available This is apparently true even of hypophysectomized animals It might be interesting to use the guinea pig as a test object to work out those conditions under which a growth hormone resistant species might be made sensitive to this principle Let me add that the same STH preparation which was ineffective in the guinea pig proved highly growth promoting in the rat under exactly comparable laboratory conditions

Conn Commenting on Dr White s hypothesis—it is true that by and large administration of growth hormone in man has not resulted in positive nitrogen balance even though the same preparations were growth promoting in animals In fact humans have frequently demon

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of the normally mobile rat skin due to this fibrous tissue proliferation

Astwood Have you not had the impression though that one preparation may differ from another in the extent to which it produces this binding down of the skin although the preparation might exhibit comparable growth hormone activities?

Selye I could not answer that because we have used only Armour s preparation for experiments in which we specifically injected at the same spot daily Unless this is done such an effect is not obtained anyway

Ralli Are there any data on the secretion of growth hormone in varying age groups? If the elderly rat were to be studied would a growth hormone assay of the pituitary indicate that it had decreased in amount? Is there any way of assaying throughout the life span the rate of secretion of the growth hormone?

Li Dr Ralli we once spent a whole year trying to detect growth hormone activity in various subjects ranging from children to people in old age without success

Long In the blood?

Li Yes these were blood studies However Dr Kinsell and I (14) made a study of patients with active acromegaly where we did find some growth hormone We still couldn t detect any in the urine but we found a high growth hormone content in the plasma

Long Is it possible to get a figure for the content of growth hormone in the rat pituitary? Is there enough there? I think that what Dr Ralli was getting at was whether there was any change with sex or age and so on

Li We haven t really tested that We have done only a few selected experiments so far That is one reason why we have tried to improve our assay method by sensitizing the animal with thyroxin

Long P A Smith placed half a lobe of pituitary under the skin of a hypophysectomized rat The next day he observed quite a sharp increase in weight

Li Growth hormone is an extremely labile protein One of the criteria of denaturation is the change of mobility in electrophoresis This phenomenon can be demonstrated by a simple experiment If a growth hormone preparation in a pH 9 5 buffer is maintained at room temperature for two days in a sterile condition there will be no change of activity but a new component having a higher electrophoretic mobility will appear

Long Would that prevent destruction of a pure form? After all tests for the principles of growth hormone are fairly sensitive

Li I don t know why growth hormone activity cannot be detected in a single rat pituitary although we can detect 5 gamma activity of

strated mild negative nitrogen balance when given growth hormone This latter finding forced us to consider the same point raised by Dr White even though administration of growth hormone produced no eosinopenia or other measurable increase in adrenocortical activity We therefore gave growth hormone to a patient with Addison s disease and failed there also to induce a positive nitrogen balance

Selye I may say that the lack of growth promoting effect observed in the guinea pig was probably not due to thyrotropic contamination in our STH The extract we used for this particular work was made by Armour specifically with the view of testing a preparation as free of thyrotropin as possible Although I cannot give exact figures, it is extremely low in thyrotropic activity

Long Dr Astwood knows as much about these new preparations of growth hormone and their lack of effect in man as does anybody It is my understanding that they did produce nitrogen retention in man

Astwood They have sometimes induced a positive nitrogen balance but not always It seems to me that growth hormone preparations vary widely in what might be called their toxicity Some of them at least in man are toxic in the usual sense of the word producing either local inflammatory reactions or fever If a preparation can produce fever in one patient it seems to me quite possible that it might be producing some noxious influence in another even though there may be no actual elevation in body temperature Increase in sedimentation rate for example would indicate an effect that is probably related to toxic impurities Raben and Westermeyer* have evidence that current growth hormone preparations can be fractionated to give apparently different chemical fractions Their work on human beings has been deferred pending further purification I should like to ask Dr Selye whether he has studied the sites of injection of large doses of growth hormone in animals whether there is not a local inflammatory reaction which varies in its extent from one preparation to the next?

Selye Our attention was called to the site of injection in those experiments in which we gave stilbestrol and STH at the same time Fibroma like local connective tissue proliferation developed at the site of STH injection Repeated injection of STH into the joint region also caused marked fibrous tissue proliferation in rats In all these experiments it was very difficult to distinguish between nonspecific local irritation some allergic reactions and specific growth hormone effects However histologically the tissue developing at the site of STH injection was almost entirely fibrous and virtually devoid of any true inflammatory component Repeated injection of STH in the same subcutaneous region for instance on the back of the animal caused a binding down

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*Raben M S and Westermeyer V W Unpublished data

Thorn Dr Robert Gaunt has made some interesting observations on the effects of various steroids in restoring the adrenal weight in the presence of cortisone inhibition*

Selye That is methylandrostenediol

Thorn That is one of the compounds There are several which are quite effective

Selye It must be remembered that methylandrostenediol causes a change in the adrenal qualitatively different from that produced by ACTH So I don t think it can be interpreted as an effect caused exclusively through a discharge of ordinary ACTH

White Regarding the existence of several kinds of ACTH (1) Sulman (15) states that evidence exists for three corticotrophins an adrenal ascorbic acid depleting factor an adrenal weight increasing factor and a chromatotropic factor What is the opinion regarding this problem? I believe that Dr Li and Dr Ingle have done work suggesting that there is a factor which lowers the adrenal ascorbic acid and that there is a separate factor which maintains adrenal size Dr Young s group at Cambridge have similar evidence I think Dr Li also has indicated that there may be a separate eosinopenic factor I wonder whether in these studies this is a dose response factor or whether the evidence is adequate that these are different substances In other words may the same substance produce a diversity of effects depending upon the dose level?

Li I must say that I was somewhat surprised to read Sulman s paper especially his remarks about the third factor I think Dr Astwood will agree that the ascorbic acid depleting factor is almost free of a chromatotropic effect or in other words that the intermedin component is not the same as the ACTH component

With regard to the adrenal weight factor and the ascorbic acid depleting factor I don t think that there is any definitive evidence to the effect that they are separate chemical components although it is suggested by evidence from chemical studies that they might be In our own experiments we found for example that when a highly active ACTH preparation let s say a preparation of a hundred units per milligram is digested with acid there is in some cases an enhancement of ascorbic acid activity but with an accompanying loss of adrenal weight maintaining activity On the other hand when ACTH is heated in dilute sodium hydroxide the ascorbic acid activity of the fraction almost disappears while the activity of the adrenal weight factor and the eosinopenic effect appear to be quite resistant to the treatment These observations are not sufficient to provide evidence for these factors

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*Personal communication

purified growth hormone by means of the tibia test It was also very surprising that we couldn t detect any growth hormone activity in the blood of young children

Thorn What are the comparable quantitative requirements in your best preparations of growth hormone and ACTH as indicated by their response in the rat?

Li We are just looking at that quantitation so to speak

Thorn Can you give an approximation?

Li No that is a very difficult problem because the effect of ACTH depends so much on the rate of absorption Apparently, growth hormone is not very much affected by this factor, in fact our experience has been that even when growth hormone was injected three or four times a day the potency of the hormone was not changed to any great extent But with ACTH it is a very different matter I do not know what the best conditions would be to control this absorption factor

Long Dr Selye outlined certain possibilities among them the possibility of a plurality of ACTHs We touched briefly before on the question of there being an eosinopenic ACTH an ascorbic acid lowering ACTH and an adrenal weight increasing ACTH Would anyone like to comment further on whether there is one ACTH or whether we have to think of its effect as due to several compounds? How about it, Dr Astwood?

Astwood I don t know We have been trying to purify what we have been calling corticotrophin, assuming that it is a single substance and so far we haven t encountered evidence that it is multiple Dr Selye s demonstration that giving a partially purified corticotropic preparation together with a growth hormone preparation caused a completely different effect upon the adrenals again brings up the question however of a second pituitary factor acting upon the adrenals

Selye I should not like to give the impression that the effect is completely different It is conceivable that the necrosis in the animals treated with both hormones was simply due to the fact that these adrenals were still more stimulated than those of the rats receiving ACTH alone and that some breakdown of the tissues resulted from overstimulation In other words I should not like to go on record as interpreting this experiment as definite proof of a plurality of ACTHs

Thorn Don t we need some reservations in this regard since we know of at least three substances which are capable of restoring the adrenal size to normal in the presence of pituitary inhibition (*a*) exogenous ACTH (*b*) growth hormone (*c*) certain steroids particularly androgenic substances

Selye I have had no experience with androgens but as regards the other two I would certainly agree

has asked about namely using different ACTH preparations These were supplied by Dr Astwood Dr Li or were certain Armour preparations and we have gone into a detailed analysis of the products by the method we described with Dr Zaffaroni Thus far we have at comparable doses seen no qualitative difference in the output of adrenal steroid no matter what ACTH preparation was used On the basis of these experiments we tend to think that whatever differences may be exhibited by these adrenotropic hormones may in some involve this phenomenon namely a tendency for production in one direction rather than another We feel this rather strongly because in examining the enzyme systems which are responsible for the production of the various transformations ending in the active adrenal steroids we find that some should be clearly labile to one type of shall we say enzyme poison and others to an entirely different set In other words the 11β hydroxylase which we have been working on very actively has certain necessary co factors for the operation of the enzyme whereas the 21 oxygenating enzyme requires quite different co factors And one can conceive of a situation where a deficit of one co factor might shift production from 11 oxygenation to 21 or even 17-oxygenation

Ralli Are these single experiments?

Pincus This experiment has been done in about fourteen beef adrenals

Bush That is red triphenyltetrazolium and not the blue anisole type?

Pincus The blue

Ralli In the work you reported several years ago if I remember correctly you gave ascorbic acid in the perfusing medium When you perfuse with ACTH now are you giving pure ACTH or are other factors included?

Pincus We have been working with ascorbic acid added to the blood and we have seen no qualitative differences in the nature of the adrenal output Mr Fish and I have found that if ascorbic acid is added the output can be sustained In other words with constant administration of ACTH the adrenal tends to decrease its output beginning at about the second to third hour of perfusion but if ascorbic acid is added the output can be maintained over a period of eight or more hours That seems to be the only effect of ascorbic acid

Ralli Have you tried any other fractions that might be part of co enzyme systems?

Pincus Oh yes we have but I do not want to give a paper now

Long I am sure Dr Ralli would like to know if you added pantothenic acid

Pincus Pantothenic acid has had no effect on the total output as far

being separate entities since they would have to be isolated in pure form before that could be stated for a certainty But evidence so far suggests at least, that there might be different activities in different preparations of ACTH

Vogt Is it conceivable to explain this type of result by assuming that the molecule has been modified so that its rate of absorption is changed? The ascorbic acid test is a very rapid one A sudden rise in blood level of ACTH and then a rapid fall is wanted On the other hand for the adrenal weight increase, I think a constant rather low level of ACTH circulating from the site where it has been injected would be desirable Is it just possible that this might explain the results?

Li That may explain the variation in ascorbic acid but in the case of the adrenal weight factor we always use a vehicle which allows for delayed absorption like a beeswax suspension in peanut oil when we inject animals to assay for the adrenal weight factor I think then that it is quite clear in this case that the adrenal weight factor is lowered as the hormone is treated with an acid and maintained at the same level when it is treated with an alkali

Long We are getting around to the point in which Dr Bush and Dr Pincus are very much interested ACTH, one or three of them is producing the effects observed by increasing or changing the rate of secretion of adrenocorticosteroids I wonder what evidence there has been on the effect of rate of secretion of adrenal steroids on these different types of ACTH preparations Dr Pincus perhaps we can begin with you and then Dr Bush can tell us his experiences

Pincus Some very recent work tends to substantiate that all ACTHs are the same but that different results can be obtained with the same ACTH In regard to the latter point we have some preliminary data from Mr Macchi of our laboratories that at a critical concentration (about 0 1 I U per liter) of ACTH in the perfusing medium a significant production of steroid glycol (or glycerol) may occur whereas at other concentrations (e g at 10 I U per liter) practically all of the steroid produced is α ketol We arrive at this notion by comparing the titer of formaldehydogenic substance in the steroid extract with the titer of α ketol measured by the triphenyl tetrazolium reaction The formaldehydogenic substance titer arises from formaldehyde generated from either ketol glycol or glycerol side chains at C_{17} whereas the tetrazolium reagent appears to be specific for ketols the formaldehydogenic substance minus the triphenyl tetrazolium reaction should therefore indicate the amount of glycol or glycerol Differences in the nature of the adrenal secretory product may arise within the adrenal itself depending on the ACTH concentration supplied to it

We have done the experiment a number of times which Dr Long

TABLE I

Influence of Different ACTH Factors on Adrenocortical Secretion in Hypophysectomized Rats

Figures are total Δ^4 3 ketosteroid secretion rates in mg /Kg /24 hrs

	GROUPS				
	I	II	III	IV	V
A) Hypophysectomized (1)*	0	0	—	—	—
1 day (2)*	0	6	—	—	—
B) Hypophysectomized (1)	0	0	0	20	0
7 days (2)	0	0	0	20	0
C) Hypophysectomized (1)	0	0	—	—	—
42 days (2)	0	0	—	—	—
	No treatment	AW factor	AA factor (aqueous)	AA factor (beeswax)	Placental ACTH

*1 and 2 refer to first and second samples of adrenal blood

weight while the placental factor had an effect very similar to the growth hormone which was to increase the adrenal weight or maintain it

Histologically these adrenals showed that those from the A W treated group were exactly the same as those obtained by Dr Renford The gland was loaded with lipid and the lipid free zone inside the zona glomerulosa was abolished In the A A treated ones there was little or no effect With A A in beeswax there was lipid depletion and great dilation of the venous sinuses of the gland the gland was hyperemic and lipid free in all the groups The placental ACTH produced effects very similar to the growth hormone but there were slight histologic differences between the effects of the placental ACTH and the A W factor

Finally the animals were anesthetized with ether and Nembutal the left adrenal vein was cannulated and adrenal blood collected for two consecutive 20 minute periods The second sample in each case was taken during stimulation with a continuous infusion over 20 minutes of an equivalent of 1 mg of Armour s ACTH

In group I category A the first sample contained no detectable steroid The second sample contained about 25 μg of compound B and somewhere about 3 to 5 μg of an unknown compound which is a Δ^4 3 ketone but does not reduce triphenyltetrazolium chloride and from certain other properties appears to be 20 21 glycol The other untreated

as we could see at least in acute experiments We have also used pantothine the presumed precursor of co enzyme A and we have seen *absolutely no effect from it* We at times obtain somewhat of an effect if we combine pantothenic acid with nor epinephrine but don t ask me why

Long What kind of effect?

Pincus There is apparently an increased amount of steroid

Thorn Where neither one alone produces it?

Pincus Yes

Long Dr Bush would you like to make some comments?

Bush Dr Li (16), Dr Selye (17) Prof F G Young and M Stack Dunne and Dr G Renford have all obtained ACTH preparations which *tend to produce a great increase in adrenal weight and excessive deposi* tion of lipids in the adrenal as distinct from the picture given by exces sive doses of ACTH preparations which are active in the ascorbic acid depletion test Professor Young and Stack Dunne have called their two preparations the adrenal weight factor and the ascorbic acid factor abbreviated as A W factor and A A factor The difficulty is that there are two possible ways of interpreting these results Dr White has suggested one namely that the various end responses measured appear at different thresholds of dosage The other interpretation brought up by Dr Vogt is that it may be due to differences in absorption rate Dr Sayers (18) *in a series of rather good diagrams showed that* with *differ ent rates* of absorption or *different quantities* and *different time rela* tionships with one single ACTH different histologic pictures and different effects on the weight of the adrenals can be expected

Stack Dunne and I thought it would be good to try to check this by a method which would overcome the criticism of differences in absorp tion rate The experiments were done as follows Table I the animals were hypophysectomized 1 7 14 and 42 days before the final experi ment The groups consisted of about 5 animals each One group was untreated throughout the whole period They were all on a bread and milk diet which was found to keep these rats in very good condition The second group was treated with the adrenal weight factor The third group was treated with the ascorbic acid factor in aqueous solu tion The fourth group was treated with the ascorbic acid factor in beeswax *to retard the absorption A placental ACTH preparation* was given two times a day 1 mg subcutaneously to the fifth group The same differences as Dr Renford described although these were hypophysectomized rats were found with these different preparations

In all these animals the adrenal weight was maintained or increased by the adrenal weight factor The ascorbic acid factor either in aqueous solution or in the beeswax medium caused little or no change in adrenal

It appears that there are two ACTHs if they can be called adreno trophic but that one doesn t seem to troph or affect the adrenal in every way it might

Pincus I should like to make one remark about this disappearance of responsivity A phenomena which we have frequently noticed in the adrenal that is being perfused is the necessity for what we call a priming action If an adrenal is mounted and perfused immediately with ACTH with the first passage of ACTH through that adrenal there may be little or no increased steroid output depending apparently upon the condition of the adrenal If blood is circulated through that adrenal either with or without ACTH for one or two cycles and then the ACTH is given an increased output is observed Perhaps your second series might change to something positive if you had some means of priming

Bush You get similar cases clinically do you not?

Thorn Yes

Bush Dr Thorn has mentioned as an aside to me that the next thing is to take the same groupings of animals and do the experiment over again but treat the ainmals for longer periods It seems that although the weight factor maintained the adrenal it nonetheless did not have the priming action

Thorn Do you think that a mixture of A W and A A would give a value of 0 35 or 70 during the initial 20 minutes of observation?

Bush I think actually the the experiment to do is to treat the rats for a week with multiple injections of ascorbic acid factor in aqueous solu tion and stop the injections immediately before the experiment so that the secretion rate is low enough during the first sampling to obtain a subsequent response to another dose of A A factor What one wants to know is whether 24 hours after stopping treatment with A A factor alone the adrenal will again respond to A A factor

Ralli Does the adrenal have to be conditioned before experiments are run?

Pincus We do it now routinely in order to get a maximum yield The yields I originally reported here ran about 2 to 3 mg formaldehydo genic substance per cycle The cycle lasted ordinarily for an hour Now our outputs run from 5 to as much as 10 milligrams In order to get up to that level we use the initial priming with blood We can never reach that high level immediately Sometimes indeed the output with ACTH is on the initial run through exactly the same as without ACTH

Ralli What explanation have you for this lack of effect of ACTH? Could it be concerned with the enzyme systems within the cells which may need stimulation before they begin to operate?

Pincus I do not have factual data but I think essentially this means that the chain of enzyme systems which must operate in order to give

groups (IB and IC) showed no steroid in the first or second samples In other words over a period of 20 minutes their adrenals not only secreted nothing initially but did not respond to the ascorbic acid factor

White Pardon me Dr Bush I am not quite clear what you mean by samples

Bush Two samples were taken from each rat In each case the A A factor was injected intravenously every 5 minutes during the collection of the second sample In some animals a possible delayed action of the A W factor was tested by injection at the beginning of the first sample

Pincus Was this continuous treatment for 1 7 14 and 42 days?

Bush Yes The group numbers refer to the treatment given between hypophysectomy and the collection of adrenal blood up to the adrenal cannulation experiment

Thorn I am not clear as to the control group during the 7 14 and 42 days What happened to them?

Bush In the untreated control groups no steroid was recovered from the first sample and no detectable steroid was recovered in groups IB and IC when acorbic acid factor was injected In other words there was an immediate and considerable response in this gland in the one day hypophysectomized group whereas in the others there was none

The peculiar thing is that although the adrenal weight factor and the placental factor maintained the adrenal weight at the normal level or actually caused an increase and produced a gland which was rich in lipids and apparently in fine condition to start secreting whenever stimulated the same behavior occurred in these A W treated animals as occurred in the untreated The adrenal weight factor maintained the weight and histologic appearance of the gland but did not maintain the ability of the gland to respond to the ascorbic acid factor

In the case of the ascorbic acid factor treated animals the ascorbic acid factor being given in aqueous solution group 1A responded normally But again there was no response in groups B and C which were hypophysectomized 7 and 42 days previously The animals treated with ascorbic acid factor in beeswax showed little or no increase in adrenal weight and depletion of adrenal lipids About 35 gamma was found in both the first sample and the second samples The secretion rate in the first sample was so large that the injection of exogenous A A factor was not sufficient to produce any effect

In none of these cases as Dr Pincus also found was there any significant difference in the type of steroid released It seems to indicate the rather curious result that there is a factor in pituitary extracts the adrenal weight factor which maintains an apparently normal gland or a gland well loaded with lipids and yet will not maintain the ability of that gland to respond to the factor which actually causes the increased secretion of these hormones

be absorbed more slowly so as to give different effects but that even if injected intravenously it may depend on dissociation for its effect

Long One question I should like to ask goes back to work by Dr Astwood and Dr Tyslowitz (19) and also to some work by Dr Sayers showing that the ascorbic acid content of the adrenal one day after hypophysectomy is around 400 mg per cent and 42 days after as I recall it is in the neighborhood of 100 mg per cent The same is true of the cholesterol content Dr Sayers and Dr White remember that in the very early experiments we did with ACTH we found in one to three days a very good response in cholesterol whereas fourteen days afterwards there was a very poor response Certainly the chemical composition of the gland is quite markedly changed the longer the interval after hypophysectomy particularly in relation to the ascorbic acid and the cholesterol content This goes back to Dr Pincus point about the requirement of certain factors in order for the reactions to proceed

Ralli The fact that certain factors seemed to be required for the reactions to proceed might be the explanation for the longer sustained output when the glands were primed with ascorbic acid I wonder Dr Pincus whether you have any data on the ascorbic acid content of the glands as they came from the slaughter house Are they very low?

Pincus Extremely 50 to 75 per cent in a beef gland

Ralli What is the concentration of ascorbic acid in the beef adrenal gland as compared to other species?

Pincus It is not as high as in the rat and perfusion does not bring it to even the lowest level in the rat e g about 200 mg per cent

Long I don t know how the animals Dr Pincus uses are killed but most times they are hit over the head with a sledge hammer and perhaps have their throats cut as well

Ralli This constitutes considerable stress

Bush I could answer Dr Long s question in part The cholesterol contents have been done in other experiments by Stack Dunne and the A W factor maintains cholesterol at high or normal levels Dr Ren ford s preparation which is very similar also maintains the ascorbic acid and the cholesterol at normal levels

Sayers In the untreated hypophysectomized rat the concentration of cholesterol remains high As a matter of fact it is higher than normal The ascorbic acid concentration falls

Vogt My information is probably not up to date but last spring when Stack Dunne (20) was reporting some of these results he was not quite sure whether the adrenal weight factor was different from the growth factor Is that point now settled?

Bush I think this is still a debatable point

the end product that we *measure requires certain co factors* and that these are perhaps exhausted during the period when the gland is out of the animal and is not being perfused These co factors then are restored by the priming blood

Bush Are you still using mainly female glands?

Pincus We are using mostly cow glands, but we have used steer glands for the same experiment

Bush Priming *is required with the female glands?*

Pincus Yes but it is my impression that the steer gland requires it even more than the cow gland Generally we get much poorer results with steer gland and I think it is essentially the situation you are talking about here that it is a phenomenon of disuse The unavailability of certain substrates may in the steer gland keep it inactive for certain enzymatic transformations

Selye As I was listening to Dr Bush the possibility occurred to me that the experiments could be explained without assuming the existence of several ACTHs Assuming that the weight factor is absorbed more rapidly and has a more transitory effect after each injection its influence upon adrenal weight may be cumulative nevertheless because the effect of each injection (hypertrophy) takes some time to vanish On the other hand the ascorbic acid factor would presumably have a prolonged and constant action only if injected in beeswax Could a dissociation of the two effects when the results of these two preparations are compared be due merely to differences in absorption rate? If so we would not have to assume two qualitatively different compounds

Bush I am afraid I did not emphasize one of the points which is in keeping with the absorption criticism because I was trying to compare this *mainly with Dr Pincus work In addition* to those groups in Table I we did give the adrenal weight factor in beeswax The adrenal weight factor and the ascorbic acid factor were given twice a day sub cutaneously and also they were given in beeswax intraperitoneally The results were essentially the same Again there was no maintenance of the ability of the adrenal to respond to the ascorbic acid factor

One criticism that still could be made on the basis of difference in absorption rate is that it might be supposed that the adrenal weight factor was the same essential active part of the ACTH molecule It might be an A A factor molecule hooked onto a much larger molecule but in order to act it must be free of that larger molecule Even though we were doing our best to get over the absorption problem by injecting the material intravenously at various *times our experiments may not* have been carried out for a sufficient length of time to enable dissocia tion of the molecule In other words the second criticism which the absorption rate critics might examine is that the material could not only

injected it failed to cause significant damage although it did cause some hypertrophy of the renal tubules and some degenerative changes in the heart Similar doses of LAP caused severe damage to the hearts and kidneys of nonadrenalectomized, sensitized rats These results are in confirmation of earlier observations by Dr Selye However when our adrenalectomized sensitized rats were maintained on larger doses of adrenal cortical extract the incidence and severity of damage to the hearts and kidneys was greater in animals given LAP than in rats which did not receive it Some damage occurred in rats maintained on adrenal cortical extract alone Our data suggest but do not prove that LAP had an extra adrenal damaging effect It is our hypothesis that rats given cortical extract in amounts just adequate to sustain eucorticalism under nonstress conditions are made adrenally insufficient by the injection of the highly toxic LAP If LAP has an extra adrenal sclerogenic effect it may be masked in the adrenally insufficient animal But it is also clear that an excess of adrenal cortical extract has a sclerogenic effect in the sensitized rat

Selye Since this is a rather fundamental point perhaps I may be allowed to refer back to Figure 1 As delineated there the stress producing agent acts upon one part of the body which we call a target area This stimulates ACTH production The ACTH produces an antiphlogistic (or gluco corticoid) action and a prophlogistic (or mineralo corticoid) action through the adrenal cortex STH synergizes the effects of the prophlogistic corticoids in the target area itself If an excessive amount of inflammatory tissue develops for instance in an arthritic joint I would not think from the evidence available to us that any type of hormone or hormone combination produces that inflammatory tissue but that stressor agents let us say formalin microbes or allergens produce it However the degree of inflammation is regulated by pituitary and adrenal hormones in the periphery Here ACTH certainly acts through the adrenal STH certainly acts directly in the periphery However a third possibility exists namely that STH may so alter the effect of ACTH upon the adrenal that an increased prophlogistic corticoid formation results but for that we have no evidence

Thorn Do you imply that the reason there is no ulceration in the tissue is that STH has no effect in the absence of disease?

Selye What the graph is supposed to indicate is that a stressor agent let us say an allergen or formalin in our formalin arthritis test acts upon one part of the body which we call a target area In a formalin arthritis it would be the joint tissue Its action upon the connective tissue of the directly treated joint is quite independent of both the adrenal and the pituitary because it will occur even in an adrenalectomized or hypophysectomized animal However the degree of the re

Long Has anybody any further matter arising out of the introduction Dr Selye gave to us this morning on the mechanisms by which the adrenal cortex produces different effects?

Sayers I should like to ask if Dr Selye has any data on the concentrations of electrolytes in the plasma of animals given combinations of cortisone and growth hormone?

Selye We have not

Astwood Might I ask if Dr Selye has any further evidence, one way or the other, regarding this effect of growth hormone in intensifying the desoxycorticosterone effect whether it is via the adrenal or directly on tissues? In other words does he feel that the growth hormone, acting upon part of the adrenal causes it to secrete a mineralo-corticosteroid?

Selye For certain actions of the growth hormone it has been possible to show they are not adrenal mediated that they do not go through the adrenal For others this is doubtful For instance he growth effect of the growth hormone has long been shown to be direct The enhancement of inflammation is also a direct effect as judged by experiments on our formalin arthritis test and the anaphylactoid edema of the rat These effects are manifest even after adrenalectomy The synergism between STH and DCA as regards inflammation is also manifest after adrenalectomy However effects upon the kidney may be in a separate category

The nephrosclerotic effect of growth hormone in suitably sensitized animals has never been seen in adrenalectomized rats indeed so far we have found no combination of cortical hormones which would restore an adrenalectomized animal so that growth hormone would again have this effect Therefore it is quite conceivable to me that the nephrotoxic effect of growth hormone is mediated through the adrenal and is due to some hormone other than cortisone or DCA since these are not capable of compensating for the loss of the adrenal Alternately this may be an effect of growth hormone upon the kidney itself a direct one not mediated by the adrenal but one which is dependent upon the simultaneous action of an adrenal hormone This hormone however is neither cortisone nor DCA

White What is the action of whole adrenal extract in these experiments?

Selye We have no conclusive evidence in that respect We did give STH with adrenal extract to adrenalectomized animals but only in nontoxic doses Perhaps Dr Ingle has something on that

Ingle We have some rather unsatisfactory data on the relationship of the adrenal cortex to the damaging effects of LAP in the sensitized rat Our first studies were on adrenalectomized sensitized rats given minimal replacement doses of aqueous adrenal cortical extract When LAP was

cold can result in nephrosclerosis and hypertension (21) I am not aware of any data bearing directly on the role of the anterior pituitary adrenal cortex axis in causing the damage which results during exposure to cold I suggest that future research might be profitably diverted from studies on excesses and imbalances of exogenous hormones under unnatural conditions to studies of the metabolic and morphologic consequences of exposure to natural stressors under naturally occurring conditions and the role of endocrine organs in these changes However for the sake of speculation let us suppose that it has been proven that exposure to stress causes adaptation diseases in the sensitized organism and that the response fails to occur in the adrenalectomized animal treated with adrenal cortical extract Dr Selye has regarded such evidence as proving that the adrenal cortex mediates the damaging effect of LAP (22)

I have made up a mechanical analogy illustrating a possible relationship of the adrenal cortical response to the damaging effect of stressors An analogy proves nothing but it may serve to clarify a meaning This particular one is inexact as it was drawn up several years ago but it will serve my purpose This is going to ruin the transactions of the Macy

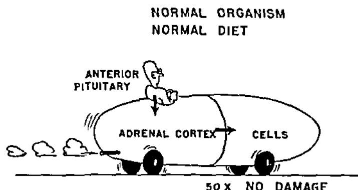


Figure 12

Conference Figure 12 represents a normal organism under nonstress conditions There is the adrenal cortex and there is the anterior pituitary which governs it We do not know the basic action of the adrenal cortical hormones so we call it X We can assume with some reason that the cortical hormones affect the rate of some basic process or processes rather than create any new reaction We represent a state of eucorticalism by the arbitrary figure of 50X Under normal conditions adaptation diseases or damage does not occur Moreover a normal organism withstands a considerable amount of stress or load (Figure 13)

sponse is largely subject to the type of pituitary and adrenal reaction that occurs in response to this local stimulation If we imitate this endogenous hormonal response by giving ACTH, we can inhibit the response to topical stress in connective tissue The depression in the surface of the skin indicates connective tissue atrophy and lack of responsiveness to topical irritation For example in the pneumoderma experiment the hydrocortisone treated region was not only unresponsive to inflammation but also atrophic and that is why there was a depression in the skin Only the prophlogistic corticoids such as DCA alone and STH alone and particularly the combination of these hormones (there is more than a summation, there is a potentiation between the two) increase the responsiveness of the tissue to any such phlogistic stressor stimulus

Thorn This diagram would suggest that STH itself does not antagonize the action of cortisone I thought that in your experiments it did

Selye Perhaps I am not expressing myself well This graph is supposed to indicate that they *do* antagonize each other Half of the drawing the right half indicates what would happen if A C say hydrocortisone or cortisone were predominant The left half indicates what would happen if P C desoxycorticosterone or some other mineralo corticoid and/or STH were predominant When both of them are present in suitably balanced amounts then nothing happens

Thorn I may be confused but I still do not understand why you have the arrow indicating STH coming down on one side of the diagram in which there is evidence of proliferation of tissue and STH not coming down on the other side with a tendency toward inhibition of the dip induced by cortisone since it would appear in the adrenalectomized animal that STH can offset to some extent the effect of cortisone

Selye Let us put it differently then It may be confusing to illustrate in one diagram, such as Figure 1 what happens when one side predominates If the target is irritated let us say with an allergen or bacterium or formalin the encapsulation and the fibroplasia in response to this agent would be greatly enhanced

Ingle I should like to pose a general question which relates to Dr Selye s discussion Dr Selye has clearly shown that these hormones given in excess or administered to sensitized rats will cause pathologic changes which simulate a number of diseases which occur in man These facts are important in providing a basis for the making of hypotheses and for further research on the etiology of diseases

According to Dr Selye s hypothesis derailment of adrenal cortical function as the result of exposure to nonspecific stressors plus certain conditioning factors can cause the adaptation diseases There are a limited number of data indicating that exposure of the sensitized rat to

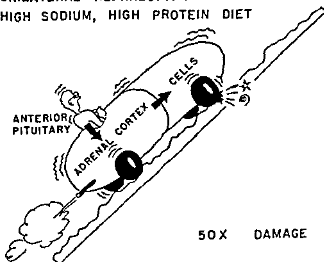


FIGURE 15

suffices to maintain eucorticalism (50X) nothing has derailed or gone awry with its function but the consequence of maintaining that same rate under increased load completes a pattern of circumstances which *results in something giving way*

It is possible to limit the production of damage by removing the adrenal cortices (Figure 16) The anterior pituitary will still pour out

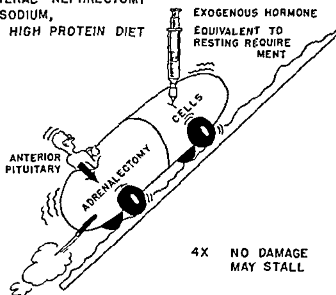


FIGURE 16

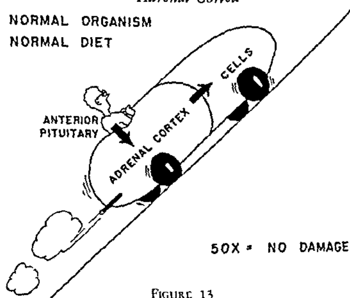


FIGURE 13

without damage although the anterior pituitary must secrete more corticotrophin and the adrenal cortex must secrete more of its hormones to maintain a state of eucorticalism (50X)

Now something is done to the organism to make it more difficult to get along, it is sensitized We can weaken the capacity of the organism to withstand stress (unilateral nephrectomy) and we can add some obstacles (high sodium chloride) However it can still get along at a normal rate (50X) without damage *if it is not subjected* to an added stress (Figure 14) When such a sensitized organism is subjected to an added stress (Figure 15) the anterior pituitary must pour out more corticotrophin and the adrenal cortex must secrete more of its hormones This response of the anterior pituitary adrenal cortex axis only

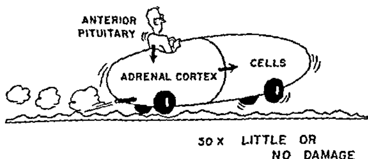


FIGURE 14

There is a difference between conditioning and permissive actions The difference as I see it and I should like Dr Ingle to correct me if I misinterpret his views is this in his terminology of the permissive action we assume that one factor say the corticoids are not in them selves doing anything, they are not in themselves pathogenic they merely permit an agent a stressor to become pathogenic In my interpretation of conditioning factors I assume that an agent in itself is potentially pathogenic but that the conditioning factor can increase or decrease its pathogenic effect In one case it is only a matter of yes or no the permissive action permits or does not permit But in the case of conditioning factors there is a matter of degree The more corticoid that is produced the more corticoid that is exogenously injected into the body the greater the number of lesions which result That is why I prefer the term conditioning Permissive to me implies that the corticoid hormones in themselves cause no lesions whatever Once there is a threshold amount which is just permissive it is assumed that the corticoids cannot do more they cannot intensify a response in proportion to their concentration in blood and tissues

Ingle That is a possible interpretation but I do not believe that we can exclude the possibility that they are the same Dr Long brought up the question as to whether a permissive effect is all-or none or whether there is a relationship between the amount of cortical hormones involved and the extent of response I should like to raise one other point Although we agree that a combination of factors is essential for the production of damage for the production of disease it is still important for us to find out in the laboratory whether or not the function of the adrenal cortex has to be derailed in order to produce the damage Is it necessary to have an imbalance in adrenal steroids as between the 11 oxygenated steroids and desoxy like compounds or an imbalance be tween certain adrenal steroids and growth hormone?

Pincus There is fairly direct evidence that in the human stress causes a differential secretory action of the adrenal I am referring to the well known urinary steroid pattern that follow stress For instance immediately following a surgical operation or a burn there is an out pouring of 17 ketosteroids which is rather transient and then a long period in which the output is very low However rather interestingly we find that the total corticosteroid also shows a rise following stress but continues to stay up In other words whatever balance 17 keto-steroid represents in the normal nonstressed individual it is certainly changed I do not think that those fundamental facts are likely to be upset except in detail I think that the 11-oxy vs 11 desoxy situation is one which will have to be determined by actual analysis We cannot speculate much on the basis of the indirect data As far as I know

extra corticotrophin but its target organ is gone If exogenous cortical hormone is given in an amount equivalent to resting requirements the organism will slow down (4X) or it may die At the slower rate of living damage does not occur

What should we call the role of the adrenal cortex in the production of damage in this situation? Is it fair to characterize it as causal? It is a cause of damage in the sense that the adrenal cortical response to stress is a necessary antecedent event but would it not be better to describe the adrenal cortical hormones as having a permissive role in causing dam age? Isn t it just as sound if not more so to reverse our terminology here and to represent unilateral nephrectomy and a high sodium load as a part of the pattern of primary causes of the damage? Can we not consider the response of the anterior pituitary adrenal cortex axis as a conditioning factor?

Selye One aspect of these drawings which Dr Ingle did not com ment on obviously represents the G A S the thing that comes out of the exhaust

I should like to put it this way as far as desoxycorticosterone pro duction is concerned I do not think it really matters whether the conditioning factors are called *the* thing and the hormone the condition ing factor or vice versa I think that in order to produce certain disease conditions a well defined pathogenic situation is needed Only if all ingredients of this pathogenic situation are established does disease ensue Moreover I doubt that one or another ingredient can be con sidered separately

For instance in the experiments to which particular reference was made DCA alone did not cause these diseases except if enormous doses were given which might well be considered to be outside of what is likely to happen normally under natural conditions Sodium chloride will not cause these diseases DCA will not cause these dis eases Unilateral nephrectomy will not cause these diseases Any combination of two among these factors will not cause these diseases such as DCA plus salt or DCA plus nephrectomy or nephrectomy plus salt If all three factors are present then the disease results very easily I think it is more or less a matter of semantics whether one or the other is called the conditioning factor If the sodium and the nephrectomy are called the conditioning factors of DCA it is no more justified than if we call DCA plus salt the conditioning factors for the nephrectomy and so on

I should like however since Dr Ingle has mentioned these factors which mutually synergize each other s effects to say a few words about conditioning versus permissive actions I missed the early part of the Conference but I understand that this was touched upon then

or the engine would freeze But the throttle and not the oil would be regulating the speed of the engine In the same way if the engine were running at 30 miles per hour and an amount of oil injected which was suitable for 60 miles per hour the engine would begin to smoke and trouble would ensue because of a number of changes in the engine s metabolism

This concept of adrenal function seems to have a better basis in fact at the moment than one postulating that the adrenal cortical hormones are equivalent to a throttle or a trigger regulating mechanism Is there any physiologic situation in which it can be proved experimentally that more cortical hormones are injected into the system than are needed relative to the speed at which the engine is going? If not then it means that parts of the body on which the stress is acting are receiving and using all the excess secretions of the adrenal and there is no reason why the heart or the kidneys or any other organ should suffer from an excess of hormones I think Dr Sayers put forth much the same argument in his article in *Physiological Reviews* (18)

Thorn We have observed marked improvement in patients undergoing bilateral adrenalectomy when a very high level of cortisone or compound F was provided throughout the operation and the immediate postoperative period The dose employed in some of our experiments has been double that which appeared to be necessary for maintenance of a satisfactory clinical condition during and immediately following the operation

Bush In other words if an Addisonian was given enough steroid to produce the same urinary increment as in an intact person his passage through the operation could be improved by doubling the dose?

Thorn Right

Bush Under that stress it appears that the normal human turns out if anything less than the optimal amount of cortical hormone?

Thorn That is true as judged by over all clinical evaluation of the patient s response although we are not yet in a position to make a critical evaluation of the specific effects of large doses of adrenal hormones on aspects of convalescence such as wound healing

White Aren t you making the assumption that the tissue responsivity to hormone is the same in the two situations or that the tissue requirements for hormone are the same? Perhaps the level of hormone required to produce a tissue response could differ in the Addisonian as compared to the normal person I believe this has been shown in normal vs adrenalectomized rats

Thorn There is no doubt that hormone may be utilized differently when first introduced in a patient with Addison s disease However I doubt whether there is any significant difference in the handling of

there is no direct analysis which would give an answer

Selye Concerning Dr Ingle s question about the derailments of the adaptation syndrome, I should like to clarify my point of view If I use the term derailment of adaptive hormone mechanisms, I do not mean to imply that this is always and necessarily due to a disproportion between pro and anti phlogistic hormones The term is used rather to denote any deviation in the hormonal response of the organism during stress which causes or permits the development of disease Looking at Figure 1 which is designed to illustrate the simplest formulation of the G A S and the diseases of adaptation concept, it can be seen that there are three major components (a) the stressor agent which is of course quite independent of the hormones and usually comes from the outside (b) the antiphlogistic hormones, and (c) the prophlogistic hormones Any disproportion between these three factors could have a pathogenic result For instance if our topical irritation arthritis experiments are used as a basis for discussion the introduction of formalin into the joint region corresponds to the action of the stressor It will cause inflammation with or without the help of endocrine glands by a direct effect upon the target area However, the course and intensity of the resulting experimental arthritis will be enormously influenced by hypo physectomy adrenalectomy or treatment with hypophyseal and/or corticoid hormone If formalin is injected into the joint region of an adrenalectomized animal the resulting arthritis is much more severe than it would be normally but its intensity can be artificially repressed by simultaneous treatment with antiphlogistic hormone (e g cortisone) The more cortisone given the more effectively can the inflammation be suppressed This is therefore not a yes or no effect It is definitely a matter of a perfect balance between the pathogen (in this case formalin) and the hormones which are needed to diminish its patho genic action It would be entirely wrong to consider that the term diseases of adaptation necessarily implies a derangement in the balance of mineralo and gluco corticoids

Bush Isn t the question actually one of whether in the normal *situation there is ever a secretion of the adrenal cortex which is greater* than that required by the particular stress What Dr Ingle and also Dr Engel have shown is that in most situations where metabolic re sponses occur to stressful situations in intact animals the responses will still occur in adrenalectomized animals if they are maintained on a con stant intake of exogenous cortical hormone but that the extent of the response is limited by the quantity In other words there is a quantita tive relation It is rather like the situation which would obtain if an automobile engine had a separate injection system for the oil If the engine was run at 60 miles per hour more oil would have to be injected

tuberculosis the dilemma occurs that if his adrenals produce enough antiphlogistic corticoids to suppress the arthritis his pulmonary tuber culosis is likely to be aggravated because here too inflammation and encapsulation will be suppressed While in the case of rheumatoid arthritis the suppression of the defensive inflammation is beneficial and does not appear to enhance the spreading of the original causative path ogen the reverse is true in the case of the pulmonary phthisis of the same patient It is hardly conceivable that the adrenals could produce just the right amount of gluco corticoids since no amount is just right for both lesions Presumably topical conditioning factors must enter the picture in order to decondition the gluco corticoid effects in one place while enhancing them in another

Long Aren t we coming to the key point in this whole discussion of your concept that the hormone blood levels as a result of a response to whatever causes rheumatoid arthritis would rise to the value at which it has been shown the spread of tuberculosis would be enhanced? Isn t that the point of Dr Bush s argument too that actually levels of blood hormone which may be necessary or are required in the damaged area have a pathological effect in the undamaged areas?

Selye I would agree

Long Dr Sayers point I think would be that this is where the self imposed regulatory mechanism would come into play and thus prevent the blood level from being increased to these extremes or at least to the point where tissues are damaged

Selye Although this may not be considered to be a separate disease accidental thymic involution is a condition whose mechanism we have studied in some detail As it is a well known syndrome in pediatrics we might perhaps use it as an example Thymus involution during the alarm reaction is certainly due to adrenocortical hyperactivity It can be produced by any stressor during an alarm reaction it cannot be produced after adrenalectomy by any alarming agent and finally it can be elicited even in the absence of both adrenals and of stress by minute doses of gluco corticoids This thymus involution is accompanied by the charac teristic hematologic changes of stress as a result of exposure to say cold formalin infections or any other stressor In this case the corti coids are produced presumably in order to enhance resistance to the general damaging effects of the stressor However inescapably the thymus responds with dissolution of its lymphoid elements which here represents an unavoidable side reaction of endogenous corticoid over production

The same could be said about inflammation An alarm reaction in hibits inflammation no matter what is used to elicit the latter Undoubt edly the production of antiphlogistic hormones during the alarm

hormone in the Addisonian who has been treated with cortisone over a long period of time Furthermore our bilateral adrenalectomized cases have their own adrenal glands up to the moment of operation

Ralli Do these Addisonians get more salt than a normal subject?

Thorn We do not use supplementary salt in the maintenance of most of our patients with Addison s disease They eat a perfectly normal diet

Long Hasn t it been shown that an adrenalectomized animal subjected to stress actually requires a great deal more hormone in order to live? There must be many examples of that in which the amount of hormone which is required for life maintenance under basal conditions is greatly increased if the animal is placed in an unfavorable environment That does not say following Dr Bush s point that when the secretion of hormone is increased four of five times the animal is subjected to an excess of the hormone

Bush Exactly We know that ten or twenty times as much cortical hormone is required to maintain the rat s work output from a stimulated muscle as is required to maintain its life However the point is does the adrenal under normal stimulation by the pituitary ever in fact put out fifty times as much instead of the required twenty times as much?

Selye When we want to apply this thought to the concept of the diseases of adaptation I think we ought to take into consideration also that both an excessive or inadequate adrenocortical response may become pathogenic If during stress the adrenal cortex did not respond with the optimum amount of corticoid production this in itself would also lead to a pathogenic situation The corticoid production mechanism would be derailed in the sense that it was inefficient and thus a factor in the causation of disease

If I might apply that to the much discussed problem of the pathogenesis of rheumatoid arthritis I would say this Although we do not know what pathogen (microbe allergen etc) is originally responsible for the production of rheumatoid arthritis it is evident that the adrenal of a patient who suffers from this disease does not produce the necessary amount of corticoids to prevent the arthritic lesions from becoming manifest The arthritis is not due in this case to an unresponsiveness of the joints because if exogenous corticoids are supplied then the joint lesions improve

Another point which I think we must take into consideration is this In the schematic drawing of Figure 1 it has been assumed for simplicity s sake that there is only one target area subject to attack by a single stressor In clinical medicine this is rarely the case If one imagines the situation of a patient who suffers simultaneously from rheumatoid arthritis and a well-controlled latent focus of pulmonary

ditions the typical nephrosclerotic corticoid overdosage picture ensues However as I said before I cannot quote any clinical case in which this relationship has been definitely proven

Sayers Isn t it true that the concept of diseases of adaptation is very difficult to prove or disprove? I do not think it profitable at the present time to continue to discuss the concept at length because of the indirect nature of the evidence One can come up with almost any answer depending upon the way the facts are juggled They can be fitted into almost any preconceived concept Advances in methodology in particular cortical steroid analysis in peripheral blood not preoccupation with a concept will help clarify our understanding of the role of the adrenal cortex in disease

Pincus I should like to second the motion very heartily We have been trying in the case of rheumatoid arthritis particularly to get some indication of the nature of adrenal corticosteroid secretion and it all depends on how the measurements are done If the total corticosteroid output from rheumatoid arthritics is measured there is no difference from that of normal subjects The average output for persons of comparable age is 4 5 mg formaldehydogenic steroids per day in a group of normal and arthritic subjects However a detailed analysis of the corticosteroid contained in the urine is done we begin to see differences But we do not know whether these differences are traceable to the arthritis or not There is a good deal of individual variation in what might be called the urinary corticosteroid pattern and thus it may be the individual and not necessarily the disease which is responsible for the variations Therefore I am heartily in favor of Dr Sayers remark We should have better information of blood levels and of the nature of the corticosteroid secretion before speculating too freely although—the contrast between the 17 ketosteroids and the corticosteroids following damage does provide some basis for speculation

I have one other piece of information which is of interest We have compared the total corticosteroid output of a group of about thirty normal individuals with a slightly smaller group of schizophrenic persons We found to our surprise that a normal healthy individual shows a very consistent output from day to day with a characteristic output level The schizophrenic however varies markedly This variation might offer a basis for theorizing but until one can separate that which is characteristic of the adrenal I think we are juggling concepts

Bush On Dr Sayers attractive theory of the homeostatic mechanism one would expect the diseases of adaptation to be impossible unless some very severe derangement of the regulatory system occurred If Dr Sayers theory held it might be expected that increased cortical secretion could occur without any rise in the blood hormone concentra

reaction plays a special role in the resistance to inflammation during stress One can hardly imagine that the supression of inflammation would not enhance the susceptibility to diseases caused by those microbial agents against which the organism normally defends itself predominantly through inflammation Therefore I think we must admit that disease can be enhanced or caused both by an increase and by a decrease of corticoid production Indeed even if the pituitary adrenal system is not abnormal and responds during stress as it should there remains the original dilemma that the body may be the bearer of two disease conditions one of which requires an increase in corticoid production and the other of which is aggravated by this same hormonal change In that case a perfect endocrine response becomes impossible and simultaneous adjustment to the two types of hormonal requirements can only occur as a consequence of some topical compensatory responses perhaps in the sense of selective conditioning

Astwood Dr Bush s question is still not completely answered without a concrete example It is a theoretical possibility but are there any combinations of two diseases where the one is benefited and the other made worse by stress?

Selye Offhand I can only think of the thymolymphatic atrophy, and you may argue that that is not strictly speaking a disease

Thorn Perhaps a patient with peptic ulcer and rheumatoid arthritis would present the combination you want

Selye It would be extremely difficult to prove in any patient for instance one with a peptic ulcer and rheumatoid arthritis or tuberculosis and rheumatoid arthritis that an aggravation of the lesion known to be normally aggravated by excess gluco corticoids was actually caused in this particular case by the defensive production of such hormones The so called Cushingoid changes which have been observed in certain cases with chronic infections in prolonged aspirin overdosage in pregnancy and so forth might be relevant but I certainly cannot give any particular instance of a patient in whom this was proven

Long As was remarked the urinary excretion of adrenal cortical hormones continues high for a long period of time After burns for perhaps two weeks the level of adrenal cortical steroids in the urine is much higher than normal It is quite significant that in that period an excess of adrenal cortical hormones if it represents an excess is apparently not associated with any deleterious effect such as hypertension or nephrosclerosis

Selye In experimental animals nephrosclerosis caused by exposure to cold has been ascribed to excessive corticoid production The increase in circulating corticoids is useful to defend the animal against the general damaging effects of cold but under suitable experimental con

the other was not In the opinion of the clinicians the normal group was quite healthy

Long Were these steroids that you determined free? They were not conjugated?

Bush They were free

Selye If the self regulating mechanism of corticoid production functioned perfectly during stress then I admit we could never expect any disease to be due to a derailment of adrenocortical hormone production because by definition this would always have to remain the same Certainly under these conditions no lesion due to an excess of circulating corticoids could be expected since such could never occur because of the perfect functioning of the feedback mechanism However, when we first analyzed the mechanism of this phenomenon which we called compensatory adrenal atrophy we pointed out that it is precisely during the alarm reaction that it fails (25) If this self regulation mechanism did function during stress not only diseases of adaptation but the entire adaptation syndrome concept would have to be completely revised One could never expect any increase in corticoid production to occur either to combat stress or to produce disease during stress Even the adrenocortical hyperfunction of the adaptation syndrome could not really take place if the self regulation were always absolutely perfect and I do not think Dr Sayers wanted to postulate that

Long He has not said that

Bush The question is whether or not there are situations in which the pathologic requirements of some stressed target area are such that there is a consistently elevated hormone level in the peripheral blood

Selye To give just one example there would not be thymic atrophy in the presence of the adrenals during stress if there were not an increased blood corticoid level

Bush I quite agree with you but as I said the only examples we know either by direct or indirect means are cases where the anti phlogistic hormones are elevated

Sayers Dr Selye I would not be quite as dogmatic about the thymic atrophy being due to hypercorticism because I always keep in mind Dr Ingle s experiment showing that in an adrenalectomized animal given a maintenance dose of cortical hormone and subjected to the stress of a fracture typical catabolic reactions occur

Dr Ingle have you noticed thymic atrophy in adrenalectomized animals given a maintenance dose of cortical hormone and subjected to stress?

Ingle We have not studied thymic response under such conditions but I believe that Dr Selye has done so

Selye Yes In an adrenalectomized animal maintained on a certain

tion In other words, the utilization rate of some particular tissue which required more cortical hormone would be increased to such an extent that the blood level of cortical hormone remained close to the normal level with the result that ACTH secretion could be maintained whereas, if Dr Sayers theory held absolutely in the event that the blood level rose there would not be a maintained increase in adrenal secretion

Pregnancy seems to involve such an actual increase in the blood concentration of cortical hormone Fifteen cases studied with Professor Pickering at St Mary s Hospital, London have shown blood concentrations of 17 hydroxycorticosterone in the range of those found in Cushing s disease Thus the increased rate of adrenocortical secretion in pregnancy indicated by clinical findings and estimations of urinary steroids is also associated with an increased concentration of adrenocortical hormone in the peripheral venous blood

Another case is Dr Nelson s (23) experience with patients with fevers of one sort or another in which compound F in the peripheral blood was measured It was found that the concentration of compound F like steroids in the peripheral blood was normal or slightly above normal until the very termination of a febrile disease but in the last two days of the disease the concentration would rise to four or five times normal Here it seems to me is another instance where a response to a pathologic situation results in an increase in the concentration of these steroids in the blood

Long What disease was that?

Bush These were terminal pneumonia cases and severe influenza and things like that

Long In view of Dr Haines (24) recent studies on the presence of these steroids in the placenta have you any idea of how the placenta may be contributing to the situation found in pregnancy? In other words is this high level found in the first second or third trimester?

Bush Most of these cases were in the third trimester

Thorn The selection of patients who are pregnant is interesting but I am not sure that it settles the argument since we have observed an increase in steroids in patients with Addison s disease during pregnancy Presumably as far as the mother is concerned the reaction might not be mediated according to the Sayers theory but from an external source of hormone

Bush In five cases that were followed after delivery the concentration was still high for several days afterwards

Thorn That is very interesting

Ralli Did these patients have perfectly normal kidneys as far as you could ascertain?

Bush I am not a clinician We did two groups one was toxemic and

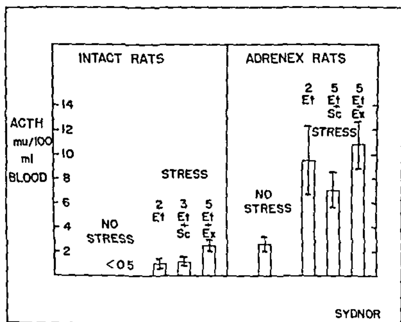


FIGURE 17 Experimental data of Dr Katherine L Sydnor of the Department of Physiology Western Reserve University School of Medicine The figure illus trates the effect of stress on the ACTH content of the blood of intact and adrenalectomized rats Et = ether Et +Sc = ether plus scald Et +Ex = ether plus exsanguination 2 3 5 indicate the time in minutes that the samples of blood were removed for analysis following the initiation of the stressful stimuli

the ACTH you infuse is being very rapidly inactivated and does not accumulate

Thorn There is no doubt that a lower dosage level would be possible if the infusion was given over a more prolonged period but if the infusion was limited to a four hour period 0 25 units per hour was the lowest level at which we could be certain of a statistically significant increase in urinary steroid excretion

Pincus That is 250 milli units

Sayers But remember the ACTH is probably distributed over the total extracellular fluid volume

Ether anesthesia for two minutes in rats resulted in ACTH appearing in the blood (Figure 17) It was in very small quantity but there can be no doubt about its presence Ether plus scald did not produce a significant increase over ether alone Ether plus exsanguination seemed to give a higher concentration of ACTH

amount of cortical hormone the thymic involution can be aggravated by simultaneous exposure to stress Conversely even without exposure to stress thymus involution is elicited in adrenalectomized animals by gluco corticoids in proportion to the amount given so that here again there is not a *permissive or yes or no effect of the corticoids* but they do participate in the production of the lesion in proportion to the amount circulating in the blood

Sayers Yes that is right You demonstrated that yourself In regard to my regulatory concept I refuse to be baited I have taken my own suggestion to heart I do not think this is the time for concepts it is a time for methodology

If the Chairman so desires I can show a slide of some observations on blood ACTH* which speak against this regulatory concept Dr Sydnor has developed a technique for the determination of ACTH in blood (26) It is essentially a modification of Dr Astwood s oxycellulose technique for the isolation of ACTH from the pituitary Modifications which Dr Bartholomew worked out in Dr Astwood s laboratory have been utilized The ACTH eluates were assayed in hypophysectomized rats by the adrenal ascorbic acid depletion technique

The results are presented in Figure 17 The blood of intact non stressed rats contains no detectable ACTH The rats were decapitated and bled from the trunk which meant that the pituitary had no opportunity to release ACTH into the circulation Exsanguination from the abdominal aorta over a period of 2 to 3 minutes resulted in a high titer of ACTH in the blood (Figure 17)

The equivalent of 40 ml of blood from decapitated rats per assay animal did not induce a significant depletion of adrenal ascorbic acid Large volumes of blood from nonstressed man and dog did not induce depletion Man and dog have more blood available than the rat Under the circumstances the amount of blood lost was relatively small compared to the total blood volume and ACTH discharge was not induced We estimate the concentration of ACTH in blood to be less than half a *milli unit per 100 milliliter* Dr *Thorn you found that a quarter of a* unit of ACTH per hour in man produces an appreciable increase in adrenal activity?

Thorn Yes that gave a reproducible increase in urinary steroids in patients with intact adrenals It was necessary to continue the infusion of 0 25 units of ACTH per hour over a four hour period however in order to observe this reaction at this dose level

Sayers That is rather interesting because the half life of exogenously discharged ACTH is about 1 to 2 minutes Therefore I expect that

*Work supported in part by a grant from the American Cancer Society recommended by the Committee on Growth

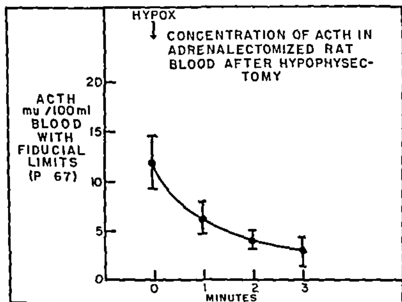


FIGURE 18 Experimental data of Dr Katherine L Sydnor of the Department of Physiology Western Reserve University School of Medicine The figure illustrates the rate of disappearance of ACTH from the blood following the removal of the adenohypophysis

end of one day? In other words are there extra adrenal mechanisms for handling ACTH which might be facilitated during a more prolonged survival following adrenalectomy?

Sayers Yes I must admit we cannot be sure that when the adrenalectomized rat is stressed the increased concentration of ACTH is actually due to an increased rate of release from the pituitary We have not ruled out the factors which you suggest a change in rate of destruction in the tissues and possibly a change in renal handling of this substance

Selye Have you studied changes in pituitary ACTH concentration in adrenalectomized animals exposed to stress?

Sayers That experiment has not been conducted

Selye That would perhaps help to clarify that point

Li I believe Dr Gemzell (27) showed that the content is increased

Pincus After stress?

Li They haven t studied animals subjected to stress

Selye If such a depletion of ACTH stores in the pituitary of an adrenalectomized animal during stress could be shown that would answer Dr Thorn s point

In the nonstressed adrenalectomized rat (decapitated and bled from the trunk so that there was no opportunity for the pituitary to discharge ACTH), the concentration of blood ACTH was relatively high This would fit with the regulatory concept that removal of the target organ and subsequent reduction in the titer of circulating cortical steroids results in an increase in titer of ACTH presumably because of discharge from the pituitary However when the adrenalectomized animals were exposed to ether to ether plus scald to ether plus exsanguination there occurred a definite increase in the concentration of ACTH in the blood over the value in the nonstressed organism

Exsanguination of adrenalectomized animals results in a marked reduction of blood volume and the increased titer of ACTH in bled rats could be due to this reduction However anesthesia with ether did not appear to reduce blood volume and hence the increased titer of blood ACTH does not appear to be due to simple reduction in the volume of circulating fluid

Thorn If a small amount of ACTH is injected into an adrenalectomized rat do high blood hormone levels result? If so is this due to inability on the part of the tissues to take up hormone rather than being due entirely to increased secretion of hormone on the part of the adrenal cortex? I should think that the threefold increase in hormone level could be more easily explained on the basis of the target organ being absent rather than on the quantitative relationship to secretion

Astwood A very small fraction of the blood would go through the adrenal in two minutes

Sayers We have no comparative studies on endogenous ACTH However in one experiment injected ACTH disappeared very rapidly from the circulation of the adrenalectomized animal Unfortunately we have no studies on disappearance of endogenous ACTH in intact rats with which to make a comparison I must admit that the type of experiment which you suggest Dr Thorn has to be done before we can get the final answer

Figure 18 illustrates Dr Sydnor's work on the disappearance of endogenous ACTH in adrenalectomized animals These animals were anesthetized hypophysectomized and exsanguinated at one two and three minutes after removal of the adrenal ACTH has a remarkably short half life From Figure 18 it appears to be between one and two minutes Adrenalectomized rats were used because they have a very high concentration of ACTH in the blood

Thorn How long had these animals been adrenalectomized?

Sayers Seven days

Thorn Do you think the curve would have been different at the

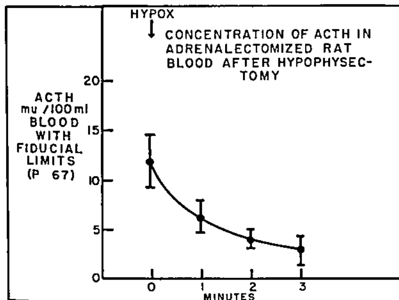


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Selye If such a depletion of ACTH stores in the pituitary of an adrenalectomized animal during stress could be shown that would answer Dr Thorn s point

Sayers It would help

Long Didn t you show that when the adrenals were taken out there was an immediate loss?

Sayers Dr Gemzell and Dr Cheng carried out experiments of this type Twenty four hours after the adrenals were removed the concentration of ACTH in the pituitary went down to 25 per cent of the normal value Then it was gradually restored to normal At twenty days it actually reached values greater than normal

We have not been too enthused about the determination of pituitary ACTH in chronic experiments, the results are difficult to interpret However Dr Selye s suggestion is a good one In acute experiments in which the animals are sacrificed two minutes after the stress is applied an appreciable decrease in the content of ACTH in the pituitary suggests an increased rate of release

Selye Another argument in favor of an increased rate of release is that if excessive doses of cortisone are administered to animals and the animals exposed to stress during that time the adrenal atrophy normally caused by the cortisone is abolished or at least counteracted by the stress Under these circumstances the adrenal will enlarge anyway although there is too much cortisone in the body

Pincus We have also attempted a study of the ACTH of rat pituitary during the administration of cortisone in doses ranging from 50μg to 10 mg per rat per day No change was seen

Conn What happens in an adrenalectomized ether stressed animal if hydrocortisone is given intravenously?

Sayers We are interested in this problem However the rat is not the animal for this study because of the limited quantities of blood Dr Sydnor plans to conduct studies of this nature in the dog In the adrenalectomized dog the titer of ACTH is very high It will be of interest to determine the effect of injected steroids on this high titer

Ralli Were these other experiments that you mentioned done in the rat?

Sayers They were carried out in the rat

Ralli Do you feel that they are a satisfactory index of ACTH activity in the blood?

Sayers It is a question of the reliability and specificity of the method for determination of ACTH in blood It is an adrenal ascorbic acid depleting method The recovery is excellent Dr Sydnor has compared the oxycellulose technique as against direct transfusion in adrenalectomized animals That is the ACTH titer in the blood of adrenalectomized animals was determined by two methods the oxycellulose technique and a technique in which the blood from an adrenalectomized rat was collected and immediately transfused into the test animal The oxycellulose

technique yielded titers of about 80 per cent of those that were obtained by direct transfusion The oxycellulose technique appears to recover most of the ACTH from blood

Long Dr Vogt remarked if I may speak for her that this rapid response in the adrenalectomized animal to ether and ether plus scald looks like the operation of a hypothalamic mechanism

Sayers Yes we have that very much in mind

Vogt What I mean to say really is that you should not feel that you are cutting the ground from under your own feet by these experiments There must be more than one mechanism of regulation

Sayers As I said before I do not think that concepts are in order at this time The oxycellulose method for the determination of blood ACTH offers new possibilities of understanding the regulation of ACTH discharge We have been dependent upon indices which measure changes in target organs hours after the pituitary has discharged ACTH Now we have a technique for measurement of ACTH titer within a few minutes after it has been discharged

Ralli With the oxycellulose method do you use whole blood or plasma?

Sayers Dr Sydnor is using whole blood A study is being made of the distribution of ACTH between red cells and plasma Until this distribution is known and until more is known about the stability of blood ACTH the physiologic work will be confined to analysis of whole blood

Thorn If you infuse blood taken from an hypophysectomized rat is any ascorbic acid depletion of the adrenal in the test animal shown?

Sayers Blood from hypophysectomized rats in a dose equivalent to 18 ml did not produce a significant degree of depletion On the other hand 1 5 ml of blood from an adrenalectomized animal gave a very significant degree of depletion

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EXISTENCE, NATURE, AND SITE OF PRODUCTION OF A SALT HORMONE (MINERALO-CORTICOID) SECRETED BY THE ADRENAL GLAND

MARTHE VOGT
Pharmacological Laboratory
University of Edinburgh

IT MIGHT BE A good idea to start this discussion by remembering why it is that doubt has ever arisen particularly in the minds of clinicians about the existence of what we term with Dr Selye mineralo corticoids since it is perfectly obvious that disturbances in the salt metabolism play such *an important role in the syndrome of adrenal deficiency* The first reason is that those steroids which are known to be secreted by the adrenal cortex and which are very active in the glycogen deposition test influence the salt particularly the sodium metabolism in the same way as the substance considered to be the prototype of a mineralo corticoid desoxycorticosterone This has been shown to be true in man and Roberts and Pitts (1) have given figures for the adrenalectomized dog which show that the same sodium retention can be obtained with cortisone and with DCA provided the dose ratio is 20 1 irrespective of the absolute dosage used I gather that Dr Pincus group in its work on the rat has found the same to be true for certain dosages although they found that if they gave more cortisone the position was different and the salt retention was changed into an increased salt excretion Thus it is perfectly legitimate to ask whether corticosterone and hydrocortisone the main known constituents of adrenocortical secretion may not fully account for its salt retaining power

Another reason lies in the fact that for many years desoxycortico sterone was assigned the role of the natural mineralo corticoid There are still workers who hold that view but there are certain difficulties about it which at least in my opinion make it unacceptable One of the difficulties lies in the toxic signs so easily produced with DCA and so rarely seen with cortical extracts It is possible that they are only the result of overdosage and not due to a qualitative difference between natural hormones and DCA but it is not too easy to reconcile the view that DCA is a normal product of adrenal secretion with the discovery by the Worcester Foundation group that the adrenal gland is capable

of 11 hydroxylating I almost feel inclined to say detoxifying DCA The main difficulty however arises from the observation that according to most authors DCA is not found in adrenal blood and that its yield from adrenal gland tissue is infinitesimal [29 mg from 1000 Kg beef adrenal according to Reichstein and Euw as cited in Thatcher and Hartman (2)] Admittedly there is one exception to these negative results in the finding by Hechter *et al*, (3) of DCA in cow blood obtained at slaughter and in adrenal blood perfused through a cow adrenal after the addition of ACTH Two facts however make one wonder whether the DCA does not only occur under very abnormal conditions as a product of incomplete synthesis First the results concerning DCA are reported not to be consistent and second the content of the blood collected from the vessels of the neck is hardly lower than that of the adrenal perfusate (120 μg against 140 μg per 2 1) I hope Dr Pincus will correct me if there has been new evidence on these points

Pincus You have stated the circumstances exactly In addition to the experiments that have been published there are some others that Dr Zaffaroni has done and he finds that the increase in desoxycorticosterone following ACTH is very small compared to the increase in every other constituent In other words whereas there may be somewhere between an eight and ten fold increase in the other constituents there is only a small percentage increase in desoxycorticosterone in the perfused gland

Sayers What about the peripheral blood?

Pincus It is extremely variable

Vogt In this connection it is interesting to remember that DCA is not the only substance known to cause salt and water retention and to benefit Addisonians the same effects can be obtained with glycyrrhetinic acid (or its glycoside glycyrrhezinic acid) through a hitherto unknown mode of action (4) Chemically this acid is sufficiently different from steroids to exclude any likelihood of its occurring in the body The suspicion that its mode of action also differs from that of the corticoids is at present based on only two observations the retention of salt and water it produces in normal man cannot be duplicated in the rat nor can the beneficial effect observed in Addisonians be obtained in adrenalectomized rats

Attempts were made by Bibile and myself (5) to test whether the activity of extracts of adrenal blood in prolonging the life of adrenalectomized rats kept at low temperature was due to the content of corticoids containing an hydroxyl group in the C_{11} position The content in 11 hydroxylated compounds was measured by their capacity to depress the eosinophils in adrenalectomized mice an effect which is as far as we know restricted to these compounds and is certainly not shown by

DCA The presence in the perfusates of a compound without oxygen in the C_{11} position but active as is DCA, in prolonging life, might have been shown in a discrepancy between assays of the same extracts of blood on the mouse eosinophils and on the rat survival time No such discrepancy was found This may mean either that these biological tests are not accurate enough for the detection of a hormone which might prolong life without acting on the eosinophils or that there is no hormonal activity in adrenal blood which is not accounted for by compounds which are equally potent in depressing the mouse eosinophils Knowing only too well the great inaccuracy of the biological tests involved I would hesitate to draw any conclusions from negative experiments such as these Assuming however the tests to be sufficiently accurate to exclude the presence of a substance lacking in effect on the eosinophils would they also exclude the existence of a separate mineralo-corticoid? I think not, because the question may be wrongly put by assuming that the mineralo corticoid we are searching for must be like DCA in all its biological properties, including lack of effect on circulating eosinophils

It seems to me that the view of the existence of a mineralo corticoid is much easier to defend if one assumes that it is not identical with perhaps not even very nearly related to DCA And in fact we have now evidence from three sides about the existence of a natural mineralo corticoid that differs from DCA The older work done on adrenal extracts culminated in the isolation by Thatcher and Hartman (2), of a compound with very little effect on glycogen metabolism but strong sodium retaining properties Its solubility in water and poor solubility in a number of organic solvents showed that it was decidedly more polar than and obviously not identical with DCA The first indication of the presence in adrenal blood of a compound with a strong action on salt retention was found in a single experiment by Spencer (6) on the adrenal effluent of the dog The work was unfortunately not followed up by the author so that the possibility that compounds B and E might have accounted for the activity was not entirely excluded More recently Tait Simpson and Grundy (7) separated a fraction from adrenal extract which travelled in chromatographic experiments at very nearly the same speed as compound E but showed a salt retaining potency of many times the activity of the cortisone present There is no doubt that this substance is neither DCA nor 17 OH DCA The authors in collaboration with Bush (8) succeeded in isolating the same substance from the adrenal effluent of the dog and the monkey so that the fact that it is a secretory product of the adrenal cortex has been ascertained Table II has been computed from their figures Shown in it is the mineral activity of steroids which they fractionated on paper

TABLE II

Mineral Activity of Steroids from Perfusate of Dog s Adrenal
Fractions Separated by Paper Chromatography

Region of Compound	Shown by Fluorescence (μg)	Equivalent Mineral Activity (as μg DCA)	Actual Mineral Activity (as μg DCA)
F	225	20	20
E	15	1	56
B	65	10	0 15

Reprinted by permission from Simpson S A Tait J F and Bush I E Secretion of a salt retaining hormone by the mammalian adrenal cortex *Lancet* 2 226 (195)

They eluted the regions of the chromatogram which corresponded to compound F to compound E and to compound B In each of these regions they estimated the amount of corticoids as shown by fluores cence and column 2 gives the figures obtained

All these compounds have a certain activity on salt retention The test which Tait and Simpson have used is actually not entirely one of sodium retention It is the ratio of sodium over potassium in the excreted urine The mineral activity of the known compounds in terms of DCA is shown in the third column It can be seen that 225 μg of compound F in the particular adrenal effluent from the dog corresponds to an activity of 20 μg of DCA since F has about one tenth the mineral activity that DCA has The activity found corresponded very well to this (last column)

The position is entirely different with the eluate of the region to which compound E travels in that the expected mineralo corticoid activity would have been 1 microgram of DCA whereas the activity found was 56 micrograms Finally in the region to which compound B travels in these chromatograms there is again reasonable agreement between the expected and actual mineral activity Therefore most of the salt retaining activity of the adrenal effluent of the dog was due to a compound eluted from the region to which compound F travels About two thirds I should say in this particular experiment of the mineral activity of the whole blood was due to that compound and only the remaining third to known compounds

White How was the actual mineral activity determined?

Vogt The mineral activity was determined by injecting the eluates from these regions into adrenalectomized mice which had been loaded with a solution of radioactive sodium and potassium and by measuring the ratio of sodium over potassium in the urine

The chemical identity of this substance is not yet established We are told that the substance is not an α β unsaturated 3 ketone but that its reaction with triphenyltetrazolium chloride indicates the presence of an α ketol grouping in the 20 21 position (9) If this color reaction may be taken as an index of the quantity present the biological activity of the compound as measured on the sodium potassium ratio of urine excreted by adrenalectomized rats is 20 times that of DCA

I do not know whether Dr Bush has any more evidence of the possible identity or lack of it of the substance with that isolated by Thatcher and Hartman

Bush No

Vogt Does it fit so far the theory that they might be the same substance?

Bush The data of Hartman and Thatcher only give very rough ideas as to its partition coefficients so that one cannot say However Dr Mason* has been doing similar work on the amorphous fraction using the life maintenance assay in which the assay for the different steroids runs very parallel to the mineralo corticoid assay as done by Tait and Simpson in the sodium/potassium ratio He has some results which correspond with these and it looks as though this compound is responsible for the high life maintenance activity of the amorphous fraction

Dr Mason is of the opinion that his substance which so far has exactly the same properties in terms of chromatographic separation and so on does possess an α β unsaturated 3 ketone group but he is not dogmatic about it I think both Tait and Simpson are doubtful about their statement that it does not contain the group and Dr Mason is also doubtful about his statement that it does I do not think that has been cleared up yet

Vogt Before I discuss the biological functions that such a compound may fulfill I should like to say a word about attempts at detecting mineralo corticoids in the urine The findings of Luetscher Deming and Johnson (10) describe a sodium retaining activity of urinary extracts of edematous patients That activity dropped suddenly when therapeutic measures provoked a diuresis and rose again when there was a clinical relapse We do not know whether there is a connection between this substance and that of Simpson and Tait Even more difficult to analyze are the findings of Stewart Robson and Tompsett (11) according to which a substance akin in its chemical properties to DCA increases in urine after salt deprivation after ACTH administration and after surgical procedures I think at present we can do no more than to register these observations

As regards the biological effects of mineralo corticoids the only point

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*Mason H Personal communication

on which there is complete agreement is that sodium retention is invariably found Potassium excretion is usually increased but the potassium metabolism is too intimately linked with the carbohydrate metabolism to produce a simple picture valid in all circumstances It seems certain that the sodium retention has both a renal and an extrarenal component The renal component was admirably reviewed at last year s Conference by Dr Pitts We are however left uncertain whether a mineralo corticoid is essential or whether the 11 hydroxylated compounds are adequate to satisfy all hormonal needs of the kidney There is nevertheless no doubt that DCA is more efficacious than cortisone in preventing renal sodium loss by the adrenalectomized animal

That there are extrarenal factors involved has been shown by a large number of observations There is for instance the fact that DCA will protect an adrenalectomized rat from water intoxication even under conditions when there is diminished water excretion so that it is within the tissues that the shift of the water from the intracellular to the extra cellular space must take place Further in the adrenalectomized nephrectomized rat DCA prevents the seizures induced by giving excess water (12) Though comparative figures are not given it is interesting to note that the same authors (13) have also shown that cortisone is more effective than DCA in protecting the intact animal from water intoxication so that here again the physiologic data do not demand the existence of a mineralo corticoid

Thorn In preventing water intoxication how long before the administration of water is hormone administered? Is the time interval of short enough duration to eliminate a renal effect as the mediating mechanism?

Vogt These are adrenalectomized nephrectomized animals which were kept alive I think for two days or so

Thorn But these animals had been treated continuously with desoxy corticosterone before they had been operated on?

Vogt No most animals were treated only after the operation

Sayers There is an important point here Desoxycorticosterone acetate has a relatively weak effect or has no effect in protecting these animals against a water load if given just prior to the administration of the water but if desoxycorticosterone acetate is given for a long period of time before the water load then it does have a truly protective effect Therefore what Dr Thorn brings up is important the time relationships in regard to injection of the hormone and the administration of the water load

Vogt And then we are confronted by the problem that the desoxy corticosterone if given a long time before might be 11 hydroxylated because apparently it is not only the adrenal which can do that

Sayers That is one interpretation Another interpretation of course is that DCA expands extracellular fluid volume and induces a hypernatremia which would provide a protective reserve of sodium in the case of adrenalectomized animals given a water load Paradoxically they do suffer circulatory collapse when given a water load and are otherwise untreated

Gellhorn A number of years ago we studied the electro encephalogram under conditions of water intoxication with and without desoxycorticosterone We found as the expression of protection which you mentioned Dr Vogt that the cortical changes were much milder in the animals treated with desoxycorticosterone They consisted in general of slow potentials of a frequency of 1 to 5 per second and an amplitude of 300 microvolts Convulsive spikes did not appear However in rats not injected with desoxycorticosterone convulsive spikes appeared singly or in groups These spikes were commonly preceded by slow waves in the normal animal subjected to water intoxication (14)

Vogt Finally the extrarenal factor is demonstrated by clinical observations on patients with renal damage In such patients large doses of corticoids raise the serum potassium instead of lowering it The simplest explanation of this paradoxical phenomenon is that the hormones cause a shift of intracellular potassium into the extracellular fluid just as they help to shift the potassium from the plasma into the urine when the kidney is not diseased These effects are observed with ACTH and DCA and with high doses of cortisone so that they do not help us much in assigning a special function to any one type of corticoid (15)

Thorn Could you tell us who it was who demonstrated the rise in serum potassium with desoxycorticosterone? There have been one or two American observers who have suggested quite the contrary that is that it is possible to lower serum potassium in the presence of renal disease with desoxycorticosterone We have not been able to confirm this

Vogt This was reviewed by Luetscher in the Ciba Conference published in 1952 It may not be his own work as he was opening a discussion

Sayers In the nephrectomized rat both DCA and cortisone increase the rate of passage of potassium into the gut lumen Despite the fact that the cortisone was probably mobilizing potassium from tissues in the nephrectomized animal the plasma concentration of potassium was either equal to or less than the control nephrectomized animals which were not given cortisone

Vogt That would be different from man

Sayers This is an extrarenal mechanism involving the gastrointestinal tract

Thorn This is apparently comparable to observations made in man Dr Steele and his group in New York and Dr Kendall Emerson in our group using resin exchange in man and in patients with Addison s disease have indicated that when the sodium content of the stool is increased with resin it is possible to demonstrate that desoxycorticosterone is followed by increased potassium loss in the stool and reduced sodium excretion

Vogt The idea that the actions of cortical hormones are intimately linked with the energy supply required for work performance by all organs of the body has appealed to many workers in the field Tentatively I should like to suggest that the mode of action of these hormones on salt metabolism is equally exerted through an effect on the energy supply to cells renal or extrarenal which perform osmotic work Such a concept does not in any way prejudice the possibility that some corticoids may act predominantly on ion exchange mechanisms and thus constitute what have been termed mineralo-corticoids

The last point I should like to raise concerns the site of production of mineralo-corticoids within the adrenal cortex You are all familiar with the hypothesis put forward by Swann (16) which suggests the zona glomerulosa as the site of production of the salt hormone Many authors (17 18 19 20) have reported atrophic change in the glomerulosa when excess sodium was fed to rats and hyperplasia when a sodium deficient diet was fed to rats This finding favors Swann s hypothesis but alternative explanations are possible Differential response to environmental conditions of the various cortical zones is not restricted to changes in the salt content in the diet Thus a high dose of estrogen administered to rats for 5 days will deplete the fasciculata of lipids but leave the lipids of the glomerulosa untouched (21) This would also appear to confirm Swann s theory but the further observation is more difficult to interpret if treatment with hexestrol is continued for two or more weeks the lipids also disappear from the zona glomerulosa Though it is I think a sound biological principle to assume that parts of an organ which show such striking differences in their histochemical responses are endowed with different functions it may be an oversimplification to assume that the task of the different layers is the production of different substances What appears quite certain is the fact recently confirmed by Miller (22) that the responses of the zona glomerulosa are not as are those of the zona fasciculata dependent on the secretion of ACTH The salt metabolism is generally thought to be less dependent on the pituitary than the carbohydrate metabolism a fact which would favor the conception of the zona glomerulosa as a site of production of salt hormone Whether however this is true in the human is anything but certain Indeed many authors have observed that ACTH has an

influence on mineral metabolism in man To cite only some examples Conn Louis Johnston and Johnson (23) have shown that ACTH has the same effect as DCA on the electrolytes of thermal sweat and this has been confirmed by Locke Talbot Jones and Worcester (24) with a different technique These authors also demonstrated that cortisone has no such effect Taking this index as a measure of mineralo corticoid activity the conclusion would be that in man at least the secretion of mineralo corticoids cannot be restricted to a part of the cortex which functions independently of the anterior lobe It may therefore be that the difference between the zones does not lie or does not only lie in the type of hormone produced but in the nature and perhaps the duration of the stimulus required for their activation

Another problem if we accept Swann s hypothesis is posed by experiments on adrenals allowed to regenerate after complete enucleation Although the regeneration takes place from the capsule and one might expect glomerulosa tissue to be formed there is no doubt that the regenerating gland produces hormones assigned by Swann s hypothesis to the zona fasciculata Moreover this occurs at a time when the serum sodium is still below normal (25) Perhaps a decision for or against Swann s theory should be withheld until instead of using DCA as was hitherto done in order to inhibit the activity of the zona glomerulosa by administering its presumptive hormone this type of experiment can be repeated with the substance isolated by Simpson and Tait One serious difficulty in the interpretation of the histologic picture lies in the *fact that the* histologists tell us that there is no cytologic criterion by which to dis *tinguish a glomerulosa from a fasciculata cell the identification of the* zones lies mainly in the arrangement of their cells and possibly in his *tochemical reactions which are reliable only in undisturbed normal* glands (e g the lack of ascorbic acid in the zona glomerulosa) Quite obviously *these problems are now ripe for direct* experimental attack by more attempts at the chemical identification of the products of adrenocortical secretion The examination should be carried out in the living animal subjected to procedures making excessive or minimal demands on the processes regulating the salt balance

Bush I think there are a few loopholes in the argument So far none of the work that has been done has actually proved that the material is a steroid The supposition that the material is a steroid is the most likely and the most economical hypothesis at the moment but it is not proven and it is only on the example of William of Occam that we adopt that hypothesis! However if we assume that the substance is a steroid then the chemical tests and the position on the *chromatogram* and the other properties which have been worked out so far would indicate that it has an *α ketol side-chain and that it has two esterifiable*

hydroxyl groups It is unstable to dilute bicarbonate solution It is extremely unstable also to dilute solutions of strong acid and the only way it has been possible to recover activity after acetylation has been to hydrolyze it by what at present is an unknown enzyme It is merely a simple esterase preparation which Tait and Simpson make up from washed red cells

Sayers Doesn t that seem a bit unusual? You would expect the acetate to be hydrolyzed in the test animal

Bush You would I agree but that is what has been found so far On the other hand the assays are done with rats while the red cells come from rabbits so that one is not bound to assume that the stuff is hydrolyzed Also it may not be hydrolyzed at a fast enough rate to be active The polarity is such that if a prolonged paper chromatogram is done it can be separated from cortisone itself it is less polar than cortisone In other words if a Zaffaroni type of chromatogram is run for 24 to 48 hours then the material is not separated at all from the cortisone If the chromatogram is run for a week until the material is right down to the front this spot is just in front of cortisone itself

They have also now several materials from Reichstein and have checked on all his substances which would be expected to flow in that part of the chromatogram none of them are identical with it That includes substance P and substance R which I think are the 3 hydroxy analogues of Dr Kendall s compound B and Prof Reichstein s substance S Isn t that correct?

Kendall I forget

Bush Perhaps these results should first be compared with Dr Wintersteiner s results (26) Or did you also Dr Kendall find that the amorphous fraction was labile to dilute bicarbonate solution?

Kendall No we have not shown that All steroids with the ketol side chain are labile in the presence of air and dilute alkali

Bush Except that the acetates of E and F can be hydrolyzed with methanolic bicarbonate and the material recovered as a free compound?

Kendall In the absence of oxygen

Bush Dr Wintersteiner compared whatever he called his compound at that stage with his amorphous fraction and he found that with the amorphous fraction under identical conditions with bicarbonate the activity was lost However the great instability of his amorphous fraction is comparable to that of Tait and Simpson s substance

Dr Mason has recently been doing similar work using life maintenance as an assay and has also found by the Zaffaroni type of chromatogram the material which he had been hunting for in the amorphous fraction running with cortisone it does appear to have an α β unsaturated ketone group Tait and Simpson say they cannot detect an

α β unsaturated ketone group but they are not yet sure of this The structure is extremely dubious and, as I say all these speculations are based on the assumption that it is a steroid

White I take it that the possibility of a compound with α β unsaturation is pretty well eliminated?

Bush I do not know Tait and Simpson have done this work largely on the basis of material extracted from glands They have tested it pretty thoroughly however I do not think it is all that clear

Vogt Is this substance stable in organic solvents provided it is protected from air?

Bush Yes In the extractions from blood I did not use any more rigorous precautions than I usually use for compound F compound B and others like that

Long Is the amount in blood increased by the administration of ACTH?

Bush That is what we are starting to determine

Thorn Can you explain why this compound does not manifest itself in the usual adrenal extracts I mean that salt retention with the potent commercial adrenal extracts available has never been a very striking feature in patients with Addison s disease

Kendall It is there

Thorn I thought you stated there is more of the compound in the amorphous fraction

Kendall Yes but that is in the extract

Thorn If it is in the extract salt retention is not very striking with the most potent adrenal cortical extracts available Do you feel that perhaps the action of a salt retaining substance is buffered by the other compounds present in the extract?

Bush Tait and Simpson have done it with their assay and found that all these compounds including E F B A DCA and this substance have an additive effect on the sodium potassium ratio That is not to say that they necessarily have an additive effect on sodium chloride excretion

Thorn No that is different

Bush But as regards the sodium potassium ratio they are almost exactly additive in their effects

Thorn It has been known for years that excessive sodium retention is rarely observed in patients with Addison s disease treated even with large doses of adrenal cortical extract This is quite in contrast with the effect observed with desoxycorticosterone

Bush Dr Kendall and Dr B B Wells showed a long time ago that if doses of the amorphous fraction were given which were equivalent to toxic doses of desoxycorticosterone in terms of life maintenance assay there was never excessive salt retention

Kendall Salt retention is raised to the normal level but not above

Sayers Dr Bush can you account for the salt retaining properties of adrenal cortical extract on the basis of the activity of, let us say the F like steroids and this steroid which is very active in sodium retention?

Bush Yes There is one thing which Dr Vogt did not emphasize and that is that in the dog experiment we also assayed all the other regions of the chromatogram including the origin and down to the front where desoxycorticosterone would have been found if it had been there The activity over all of the rest of the chromatogram was less than a 0 5 microgram equivalent of desoxycorticosterone per centimeter It would be somewhere less than a microgram per 3 or 4 centimeters of the chromatogram out of a total chromatogram of 45 centimeters

Tait and Simpson also showed that the same thing holds true for adrenal extract namely that the sum of activities obtained from the known steroid and this compound after fractionation on a chromato gram adds up to the total activity of unfractionated adrenal extracts Therefore there is no need to postulate any other highly active mineralo corticoid in adrenal extract or in the adrenal blood experiments we have done so far

Sayers No but as I see it the problem that must be answered is the one which Dr Thorn posed why is there not excessive sodium retention when large doses of adrenal cortical extract are given?

In some work that Dr Woodbury and I carried out a few years ago we were interested in the problem of potentiation or antagonism of cortisone and DCA We did find antagonism between cortisone and DCA when the animals were given a sodium load The hypernatremia in animals given the combination of DCA and cortisone was less than in animals given DCA alone I want to emphasize that the dose of cortisone in these experiments was relatively small

Vogt You are talking though of animals which have their adrenals is that right?

Sayers Yes these animals had intact adrenals which complicates the picture

Vogt These assays are done entirely on adrenalectomized animals In them salt retention occurs readily whereas it is much more difficult to get salt retention by whatever means in an intact animal

Sayers I realize that this is a complicated problem A patient with Addison s disease has difficulty not only in a situation where he is de prived of salt but also in a situation in which he is given a sodium load This is something we should keep in mind in an understanding of the whole problem that is for the over all picture of the combination of steroids and their effect on electrolytes

Ralli There is also the point that Dr Pitts made last year which was

α β unsaturated ketone group but they are not yet sure of this The structure is extremely dubious and as I say all these speculations are based on the assumption that it is a steroid

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order to determine its chemical properties At the moment these specu lations are based on amounts which even he would hesitate to use for chemical work

Li Is it the same compound that Leutscher at Stanford reported in the urine?

Vogt That is what we do not know

Bush It is quite possible There are several groups in Germany which have been working on this matter but they have all been using the benzene fractions from benzene water partitions of urine extracts

Selye You are thinking of Weissbecker and Staudinger

Bush That is right

Vogt There is no evidence that their substance comes from the adrenal and there is no evidence that it varies if either ACTH is given or if the subject is under different conditions The only criterion is that chemically and in its solubility it behaves like desoxycorticosterone acetate I think this is really very difficult to interpret

Bush You saved me from having to be rude However the thing about that work as you have emphasized is the elementary supposition that desoxycorticosterone is the mineralo-corticoid secreted and that it will be in the benzene fractions All the past attempts to detect mineralo corticoids in urine by taking benzene fractions after benzene water par tition automatically have missed this compound because the material has a polarity similar to the gluco-corticoids and would not be separ ated from them by simple benzene water partition

Long Have you made an estimate in the adrenal venous material of the proportion of this material as compared to the other steroids?

Bush If you will allow me our economical assumption I worked it out and it is rather less than one part of this substance to 100 parts of compound F in the dog

White If one more if can be postulated namely if this compound is a steroid in view of its chemical behavior and polarity I wonder whether Simpson and Tait have had an opportunity to test either com pound V or the compound N of Reichstein?

Bush Compound V?

White It is the allopregnane tetrol namely allopregnane 3(β) 11 (β) 17α 21 tetrol 20 one

Bush Related to E or F?

White It is related to F it has an 11 hydroxyl group

Bush I think they have tested substance N I do not think they have tested the substance related to 17 hydroxycorticosterone

White In any event I do not think the polarity data would fit

Bush Oh no it couldn t be Actually this flows behind 17 hydroxy corticosterone They have bioassayed compounds N R and P which I

that adrenalectomized animals have considerable difficulty in handling acid loads It seems hard to separate these various functions of the kidney into the handling of sodium or potassium or some other fraction and it would seem that there was some over all disturbance in the absence of the adrenal gland as far as the kidney is concerned

Sayers It would be very interesting it seems to me, when you are able to isolate more of this factor which causes sodium retention to study its effect on sodium retention under conditions of sodium loading

Vogt There is no evidence that this substance causes sodium retention in the normal animal That is important

Long It is different from DCA then

Vogt I would not say it does not but so far there is no evidence That I think is a point we have to be careful about when drawing conclusions

Thorn That may be merely quantitative

Vogt It may be

Thorn In man one can demonstrate with certain doses of compounds E and F under certain circumstances increased excretion of sodium and in other circumstances slight sodium retention However with large doses of these substances intravenously as a continuous infusion there may be tremendous sodium retention equivalent in every way to that observed with desoxycorticosterone

Bush Yes but Drs Kendall and Wells did try with something like twenty times the equivalent dose of desoxycorticosterone and found that it would not raise the serum sodium concentration of the adrenalectomized dog above the normal level whereas of course at that dose desoxycorticosterone would have killed the dog quite rapidly

Long Did Hartman show sodium retention in the blood with his so called sodium factor or was it only in the urine?

Kendall I think it was just in the urine It was transient too

Vogt I think he did both However these of course are adrenalectomized animals

Long Dr Bush has this compound any C_{11} activity so far as glycogen deposition is concerned?

Bush That has not been tested

Sayers If it is a steroid have you thought about explaining its relatively high degree of polarity? Presumably it does not have a hydroxyl group at position 17?

Bush We do not know that

White Wasn t the infrared spectrum done?

Bush The amount available was only one quarter of that needed for the infrared using the best microcell available Work is going on now and Professor Reichstein is trying to isolate much larger quantities in

mineralo-corticoid output has been the statement that although hypo physectomized animals and cases of Simmond s disease do not show the excessive salt loss that is shown by the Addisonian or the adrenalecto mized animal nevertheless both sets of patients and animals show exactly the same deficiency as regards the excretion of a sudden water load or resistance to water intoxication as do adrenalectomized animals *It seems however that in hypophysectomized animals and in Simmond s* disease salt retention is adequate even though one function which de pends upon the adrenal the ability to respond adequately to a sudden change in water or electrolyte concentration of the body is definitely impaired We class both phenomena under electrolyte and water metabolism but they are not necessarily influenced by the same type of adreno cortical hormone It seems better therefore not to assume that this substance is the predominant one with regard to all the processes in volved in electrolyte and water metabolism just because it makes up two thirds of the total mineralo corticoid activity in adrenal venous blood in one form of assay

Thorn I say amen to that It is very exciting that a substance of the salt retaining capacity of this substance has been demonstrated in the adrenal venous blood However I do not think one can attempt to interpret the causes of salt retention under various conditions of adrenal activity for as I said earlier substances such as compound E and F under certain circumstances can be shown to have a profound sodium retaining capacity in intact man Thus it seems possible that when the adrenal is stimulated excessively salt retention can be explained satis factorily by the large output of substances such as E and F However since in most instances sodium balance is maintained without any evi dence of overactivity of the adrenal such as Cushing like syndrome we must conclude that substances more active in their salt retaining capacity than E or F must normally be secreted When assaying bio logically salt retaining factors obtained from extracts one must be very careful as quite different results can be obtained with the same material depending upon the route of administration and the interval and time of administration Thus if compound E or F is given by mouth there may be minimal salt retention whereas the same quantity of hormone continuously infused intravenously may give profound salt retention It would appear to me that the adrenal secretes multiple hormones many having salt retaining capacities of various degrees which may in part by modified by the state of organism

Vogt May I ask one more chemical question? Is the assumption that one is dealing with a very small quantity of material which has large effects entirely based on the intensity of the triphenyltetrazolium reaction or is there other evidence? Is it possible that the triphenylte trazolium evidence is misleading?

think are the 3 hydroxy analogues of compounds A B and S All those have been found not to be active

Long Is it not active on the carbohydrate and protein metabolism?

Bush No One problem arising from these results is that two thirds of the salt activity in this assay of adrenal venous blood in the dog was due to what chemically amounts to about two thirds of one per cent of the known active steroids detected In other words one has to talk about this material as if it were relatively specific as a mineralo corticoid The amounts of compounds F and B in the blood when converted into cortisone equivalents using the data of Pabst *et al* for the relative glycogenic activity and compared with Dr Vogt s figures (27) for the amounts of biologically active material in adrenal venous blood are quite sufficient to account for the biological activity found there In other words there is no necessity to postulate the existence of any other gluco corticoid in the adrenal blood in addition to those which are known chemical compounds

Thus it seems that this substance which is present in about two thirds of one per cent of the total chemically detectable material is responsible for two thirds of the total salt activity and little if any of the gluco corticoid activity However although the lack of gluco corticoid activity may make this compound specifically a mineralo corticoid one has to guard against saying that as regards the adrenal secretion it is the specific mineralo corticoid Apart from the fact that the steroids mainly responsible for the electrolyte balance of the extrarenal tissues may differ from those responsible for the electrolyte metabolism of the kidney tubule there is also the possibility that different types of elec trolyte metabolism may be affected by different steroids Therefore although compounds E and B make up only one third of the so called mineralo corticoid activity of the adrenal blood on the basis of this assay in certain situations these compounds may be none the less more important than the so called mineralo corticoid This possibility is in dicated by the experiments of Danford *et al* (28) which are extremely interesting because they could be taken as strong evidence for the unitarian hypothesis They showed that rats put on a low sodium diet for some time apart from showing a rise in 17 ketosteroid excre tion also showed thymus atrophy of just the kind caused by the so called gluco-corticoids or by injections of ACTH Thus whether or not the secretion that was required to meet the stress of salt restriction was a gluco corticoid there was a hypersecretion of gluco corticoids and they rather than the mineralo corticoid if the rat secretes such a substance may have been responsible for combating the stress

One of the arguments against the assumption of what Dr Sayers called the autonomous concept of adrenal regulation as regards

steroidogenesis We suggested two paths of synthesis one a 17 desoxy group and the other a 17 hydroxy group progesterone being presumably the key precursor

The dotted lines in Figure 19 indicate conversions of which we had no definite evidence on the basis of isolation of the indicated compounds whereas the solid lines indicate definite isolation and identification There are obviously a number of gaps in this diagram but since it was drawn up we have managed to test a few of them The principal one is the question whether cholestenone is a possible precursor of any of these steroids

When radiocholestenone which was labeled at C_4 in ring A was perfused without ACTH and with ACTH through a battery of beef glands the counts on compound F and compound B which are always the principal corticosteroids found in these perfusates were zero There was the usual increase in total output of F and B after ACTH* This suggests that at least under the conditions of perfusion that we employ the F and B cannot originate from cholestenone So that part of the diagram can be more or less discarded

Drs Stone and Hechter have reinvestigated the cholesterol conversion about which Dr Hechter spoke at the last meeting You will recall that we were very much interested in where ACTH exerted its activity in stimulating synthesis from cholesterol Although we stated our belief that the action of ACTH must be involved in the degradation of cholesterol to some pregnenolone or progesterone like substance this was pure speculation We have some data now which makes it somewhat less speculative data on what happened when radiocholesterol was perfused without ACTH and with ACTH Here again in terms of absolute weight of the principal compounds F and B there was the increase that would be expected Also the specific activity increased in the presence of ACTH Without ACTH a total of 298 counts per milligram per minute was observed in F plus B whereas in the presence of ACTH with the same amount of cholesterol 7900 odd counts were obtained a very large increase indeed roughly an increase of 25 times in the total radioactivity

In another experiment which I think is useful in the study of the scheme of corticosteroidogenesis Drs Stone and Hechter perfused progesterone labeled at C_4 through isolated bovine adrenals without and with ACTH The amount of radioactive F and B obtained without ACTH was 0 31 mg and with ACTH 0 89 mg but the total radio activity increased from $2\ 12 \times 10^3$ to $2\ 42 \times 10^3$ or less than 15 per

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*Stone D and Hechter O Unpublished data

Bush It is entirely possible that it is misleading The activity is again based on the economical assumption that it is an α ketolic steroid One is certainly not dealing with very large quantities of a colorless material plus a small quantity of impurity which reduces triphenyltetrazolium However, it is entirely possible that the activity is not possessed by the reducing substance but by some other substance

Kendall This year I gave a paper before the Graduate Fortnight of the New York Academy of Medicine At that time I said that the investigation of the amorphous fraction was slowly gathering momentum After hearing Dr Vogt and Dr Bush and adding other information which I have obtained by way of the grapevine I would say that today the momentum was rather great and progress undoubtedly will be rapid It is not being investigated on a small scale here and there but by a concerted effort

Thorn Are we going to have any discussion about the adrenal androgens? Is there any interest in the subject?

Long I see no reason why there shouldn t be I provided an opportunity by saying that we were interested in the nature of the adrenal cortical secretion

Pincus I happen to have some slides which bring up to date the work that we have been doing on the nature of the adrenal product as studied by perfusion

Figure 19 has been presented in the past as a scheme of cortico

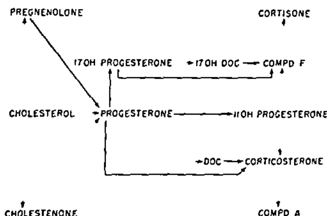


FIGURE 19 Tentative scheme of corticosteroidogenesis wherein cholesterol is assumed to be the primary steroid precursor The solid lines represent reactions which have been demonstrated the dotted lines represent possible reactions which have not as yet been demonstrated Reprinted by permission from Hechter O *et al* Nature and biogenesis of adrenal secretory product *Recent Progr Hormone Research* 6 215 (1951)

cent Moreover 5 ketolic companion substances observed in the chromatograms which increased from 0 90 before to 1 44 mg after ACTH had corresponding counts of 10 6 x 10^3 and 12 8 x 10^3

Keeping in mind the fact that we elute these from papers that may introduce materials which affect the count the over all picture is fairly clear that *is the enormous increase observed when cholesterol* was used as the precursor was not evident when progesterone was used as a precursor in the presence of ACTH This suggests then that ACTH does not act particularly in the conversion of progesterone to the products thereof in a progesterone perfusion

Another interesting feature of these data is that every single one of the five unidentified ketols is radioactive after the administration of radioactive progesterone which again points to progesterone as the probable precursor of the array of ketols that we have previously discussed Thus we come back again to our original supposition that the action of ACTH is somewhere back of progesterone presumably concerned with the degradation of cholesterol

For those of you who want more classical evidence about the nature of the products Figure 20 represents actual isolations and identification by infrared and ultraviolet of compounds appearing in a single experiment in which 550 mg of progesterone was perfused through four adrenal glands for 6½ cycles in a little over three hours from an aliquot equivalent to 500 milligrams Dr Levy and his colleagues have isolated all of these compounds from a single perfusion This is quite a feat

It will be noted that the ratio of F to B is not necessarily one to one In Figure 20 the ratio is something like 4 to 1 or 3 to 1 There is more of F In addition 11 hydroxyprogesterone has been obtained A reduction product of 17 hydroxyprogesterone has been obtained 17 hydroxyprogesterone itself and other reduction products including the 3 ketone as well as the 3 hydroxy derivatives The configuration at C_5 is always the allo configuration We have never yet seen any compounds with the normal configuration at C_5 in any of our preparations

White Have you done this with fewer cycles with the thought that you might perhaps get a clue as to the sequential order in which the various compounds may be formed?

Pincus With radioactive progesterone we have some single-cycle data

White What do you find?

Pincus Unfortunately we are now depending upon paper analysis and until we really get that straightened out I can t give you the picture The thing that always surprises us is whether we use 6 cycles or as in one of the previous experiments 3 cycles compounds F and B predominate The other compounds tend on the whole to be present

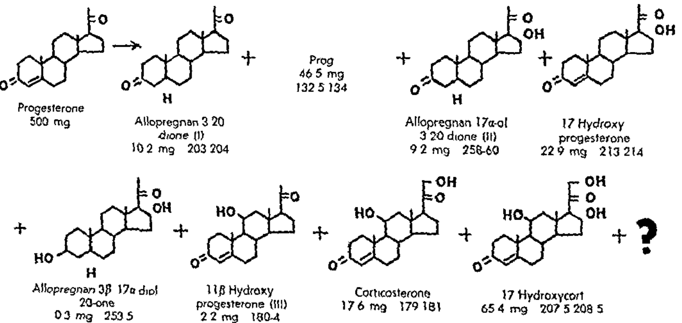


FIGURE 20 Perfusion of progesterone 550 mg 8 liters of whole blood venous flow through 4 lacerated adrenals 6½ cycles in 3 1/6 hours All compounds except I II and III have been isolated from adrenals Based on unpublished data of Levy Jeanloz Marshall and Jacobsen

rats sheep and cats there is a relatively small amount of a Δ^4 3 keto-steroid which is very similar to 11β hydroxyandrost 4 ene 3 17 dione but which at present seems more likely to be 17 hydroxyprogesterone It is found only in very small amounts and the identification is by no means complete

In three cases of adrenal tumors which I was lucky enough to have a chance of studying with Dr L R Broster who was operating on them we took adrenal venous blood from the left adrenal vein during the operation for removal of the tumors and we found apparently the same substance in each case The amounts were very small in two of the cases suffering from Cushing s syndrome with little virilism In the third however a case of long standing virilism with no signs of Cush ing s syndrome large amounts of this substance were found and the amounts of 17 hydroxycorticosterone were extremely small the reverse of the picture in the other two cases Dr J Jailer* has speculated that 17 hydroxyprogesterone may be the androgen or the androgenic pre cursor in adrenal virilism on the basis of metabolic studies which he has done and because of the fact that on the basis of the Worcester group s metabolic scheme for the synthesis of corticosteroids in the adrenal it could be supposed that adrenal virilism was some form of inborn metabolic disease of a comparatively simple kind causing the course of steroid synthesis to stop at that stage It would explain the production of this compound the tendency of these patients to have Addisonian symptoms and also the overstimulation of these glands But there is some difficulty over this theory at the moment because although I think Dr Wintersteiner first reported that 17 hydroxyprogesterone was androgenic there is now doubt about this I gather that you Dr White have found that it is active by oral administration in the rat is that right and not by the comb test or am I misquoting you?

White Not entirely It is a good example of how impressions be come information though!

Bush I can show you a letter if you want me to

White Oh no not at all I shall present what information I have Only recently has 17 hydroxyprogesterone became available in large quantities and there are several biological studies in progress including an examination of its possible androgenic and progestational activity Of the data which are completed Dr Gassner has studied the effect of 17 hydroxyprogesterone by inunction on the chick comb The steroid was weakly active as an androgen The compound was also assayed in chicks by the oral route at a dose of 15 mg per kg of food comb weight was increased about 50 per cent over the controls Androstene

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*Personal communication

in smaller amounts The only substance other than F which is rather large in quantity is the 17 hydroxyprogesterone The amount given in Figure 20 is the largest amount of 17 hydroxyprogesterone in percent age yield that we have ever obtained in any experiment In previous experiments, we obtained only a few milligrams for example in a 3 cycle experiment Whether we can by single cycles get indications of a stepwise change is something in which we are very much interested for that we are going to depend on the radioprogesterone studies I think for the isolation studies in order to get an adequate amount of any product we have to recycle at least a few times

That is about all there is to add at present to what we have reported over the past few years I think that slowly but surely we shall be able to identify all of the compounds coming after ACTH In my opinion the most interesting thing we can do is to see what kind of check we can find between those compounds and what happens after progesterone is perfused So far of the 15 regularly obtained, we are certain of only 7 We still have 8 more to worry about

About the biological activity every ACTH perfusate that we have used has sodium retaining activity We don t know where the sodium retaining activity lies because we haven t come to the point yet of analyzing for the sodium retaining factor

White I should like to make a few comments that relate to 17 hydroxyprogesterone and also to the question of what the urinary keto steroids really represent in terms of androgen production by the adrenal Dr Joseph Jailer at Columbia has had the opportunity of studying a number of cases of adrenal virilism and as you know if cortisone is administered to these individuals there is a marked suppression of the output of ketosteroids Dr Jailer thought that it might be possible that 17 hydroxyprogesterone contributes to urinary ketosteroids and in this connection I was interested that so relatively large an amount of 17 hydroxyprogesterone turned up in Dr Pincus perfusion studies

Dr Jailer obtained a base line for excretion of 17 ketosteroids in his studies of adrenal virilism He then administered cortisone and obtained the expected decline in 17 ketosteroid excretion While continuing the cortisone administration 17 hydroxyprogesterone was also given Under these circumstances there was a marked rise in the 17 ketosteroid excretion If one can make the long jump it suggests that a C , corti coid can contribute to the 17 ketosteroid excretion Dr Bush I wonder if you would be good enough to tell us about the observations which you talked about at the Laurentian Hormone Conference a few months ago

Bush The observations are these In the adrenal venous blood of

his colleagues carried out similar experiments in rats and have recently reported their results which confirm ours in all details

Sayers The possibility exists that the cortical steroids are degraded to 17 ketosteroids in the organism However I think we should seriously consider some of the degradation as being due to manipulations of the chemist after the urine is collected Mr Glenn and Dr Nelson of the University of Utah have shown that if urine is handled very gently with glucuronidase hydrolysis and then subjected to chromatographic separation an increase in 17 ketosteroids after the administration of ACTH is not obtained They had one patient a castrated male who excreted no 17 ketosteroids by their technique but did excrete C 21 17 hydroxycorticoids And they had patients with Addison s disease who secreted 17 ketosteroids but no C 21 17 hydroxysteroids

Thorn Were the Addisonian patients males or females?

Sayers These were male Addisonians If urine is treated by the routine acid hydrolysis technique then an increase in 17 ketosteroids does follow ACTH administration Part of this increase may be due to conversion of C 21 steroids to substances which give a color in the Zimmerman reaction

Sayers This does not however explain the difference between oral and parenteral administration I should like to hear some other comments about liver dysfunction and 17 ketosteroid excretion

Lukens I should like to include this question As I understand it ACTH causes a marked increase in urinary corticoids of the E type in patients with Cushing s syndrome Does ACTH cause a disproportionately large amount of 17 ketosteroids when given to a patient with virilism?

Bush I think the answer is yes That was done by Albright and Wilkins

Sayers Dr Bush shouldn t that be reinvestigated with these newer techniques?

Bush Do I understand that both methods have been done on the same urines of patients treated with ACTH?

Sayers No Mr Glenn took normal urine and divided it into two parts To one he added compound F and acid hydrolyzed both samples An apparent increase in 17 ketosteroids occurred after the addition of compound F This increase was due to background color development and disappeared when the Allen correction was applied In most hospital laboratories this correction is not applied and at least part of the ketosteroid color is contributed by acid breakdown products of cortical steroids

Bush That is interesting because it is a difficult question to settle due to the different types of conjugates split by acid and enzymic hydro-

dione 3,17 at this dose level increases comb weight by 340 per cent We have no data in rats at this time

The statement is made in the literature (29) that this compound has been shown to be an androgen and lacks progestational activity Farlier in our discussions Dr Pincus said, I believe that after a severe trauma *such as burn there is an initial very marked rise in 17 ketosteroids followed later by an increase in corticoids* One might ask whether in *view of all that has been said 17 hydroxyprogesterone is not important* in the genesis of adrenal cortical steroids With a sudden activation of the hypophysial adrenal cortical mechanism certain of the adrenal cortical steroid intermediates might escape from the gland Since Dr Jailer s work indicates that 17 hydroxyprogesterone may contribute to 17 ketosteroid production the data again emphasize the caution which should be exercised in interpreting 17 ketosteroid production as evidence of androgen secretion

Conn I should like to agree with what Dr White has just said and to recall some of the data presented at this Conference last year in this connection We had found that if one gives free compound F to normal people by mouth there is a very large excretion of 17 ketosteroids in the urine whereas if the same amount of free compound F is given intramuscularly this rise in 17 ketosteroid excretion does not occur *The over all metabolic effect of compound F by the two routes was comparable We then went to compound S which we found* to be completely inactive from a metabolic point of view But when we gave free compound S by mouth there was a four to five fold increase in 17 ketosteroid excretion whereas when free S was given intramuscularly the rise of 17 ketosteroid excretion failed to occur On the basis of these findings we made the assumption that when these precursors of 17 ketosteroids which are not androgenic come to the liver in high concentration via the portal system *the conversion to 17 ketosteroids* is likely to be intense and further that this conversion appears to be a hepatic function

Therefore we went on with this type of study in patients with hepatic disease and we found that in patients with chronic liver disease this conversion was greatly impaired When we gave large amounts of compound S or *compound F by mouth to patients with hepatic disease* we *obtained very small increases in* 17 ketosteroid excretion as compared to the normal individual More recently we have done multiple studies over a long period of time on a patient gradually recovering from an intense attack of acute hepatitis We obtained the same results in the initial stage that we had observed in *the chronic hepatitis cases But as* progressive recovery ensued *the ability to make this conversion* to 17 ketosteroids gradually returned Following our report Corcoran and

ketoetiocholanolone arise from both E and F But from adrenosterone 11β hydroxyandrosterone and 11 ketoandrosterone are obtained as well as the 11-oxygenated steroids in the etiocholane series

Kendall Is the oxygen present as a ketone or a hydroxyl group?

Pincus From E and F there is more of the 11β hydroxy 17 keto steroids but lesser amounts of the 11 keto derivatives have also been isolated This suggests that the 11 oxygenated metabolites in the andro stane series are only minor derivatives of E and F and points to a com pound like adrenosterone as the major source The point is that from E and F one does not get such 11 desoxy ketosteroids as androsterone and etiocholanolone In other words it looks as though the capacity to remove the 11 oxygen function is very limited in the human If it occurs at all the amounts are so small that they cannot be isolated Dr Thorn has given heroic doses 500 mg a day of the substance to patients and yet no measurable amounts of 11 desoxy ketosteroids have been found So what is the precursor of etiocholanolone? Etiocholanolone as well as androsterone arise from Δ^4 androstene 3 17 dione which has been isolated from adrenal tissue

In the case of Dr Jailer s experiments unless he can identify the specific substance you see the difficulty that one gets into in terms of the true 17 ketosteroid compounds in human urine Because in human urine there are roughly equal amounts of androsterone and etiocholano lone but lesser amounts of the 11 oxygenated C_{19} steroids the available evidence would suggest very strongly that the urinary 17 ketosteroids don t have a single precursor but maybe five or more

Thorn That brings up another point which has not yet been dis cussed that is the varying degree of biological androgenicity of the 17 ketosteroids as they are derived from these specific substances These analyses are time consuming and technically difficult to carry out We have had the help of Dr Paul Munson at the Harvard Dental School and have been able to make some progress in this direction One must select arbitrarily a biological assay for androgenicity I was wondering during the discussion of 17 hydroxyprogesterone whether such activity may show up in the comb test and how these assays correlate with what happens when the material is given to a normal female patient We can state on the basis of our experiments with Dr Munson that compound F gives rise to 17 ketosteroids and that these 17 ketosteroids have good androgenic potency as measured by the comb test Furthermore the quantity of androgen biologically assayed derived from compound F is much greater than that from an equivalent quantity of corticosterone

White I seem to have missed something I am not quite clear as to how these data are in harmony with the information given by Dr Pincus

lysis When he added compound F to the urine did he hydrolyze both urines with acid?

Sayers Yes

Bush How was the 17 ketosteroid determination done and was an absorption curve done?

Sayers Yes When the Allen correction is applied added F does not result in an appreciable increase in the 17 ketosteroid fraction

Bush That is quite interesting because Dr Lieberman* has recently boiled compound P with hydrochloric acid under the conditions for the urinary 17 ketosteroid determination and found no production of 17 ketosteroids at all They have done it at Utah with compound F and found that although a certain amount of dirty color is shown with m dinitrobenzene on testing the absorption curve it is not that of a 17 ketosteroid

Sayers Incidentally the paper of Holtorff and Koch published in 1940 (30) pointed out that the Zimmerman reaction is not specific for 17 ketosteroids Substances such as compound A give a color reaction which corresponds to about 20 per cent of the value that would be expected of a 17 ketosteroid

Pincus We have some information on this First of all after glucuronidase hydrolysis 17 ketosteroid is obtained

Thorn Do you mean an increase with ACTH?

Pincus No in the urine of normal persons That 17 ketosteroid is quantitatively not comparable to the amount obtained from the aliquot of the same urine which has been acid hydrolyzed

Sayers Dr Pincus did you perform a chromatographic separation of the 17 ketosteroids and the cortical steroids?

Pincus Yes these were all separated

The second point that I should like to make about Glenn s work is that he should identify the substances He should separate them Dr Dorfman and Dr Savard in our laboratory have been studying with Dr Thorn s help the metabolism of compounds F E adrenosterone and S and examining the 17 ketosteroids which arise in the urine following the administration of these materials This is on the basis of isolation work not by any other method In other words the compounds are isolated

After the administration of E and F but not after S 11 oxygenated 17 ketosteroid is found This of course is what would be expected and it is nothing unusual But the interesting feature of the data is that after F the 11 oxygenated 17 ketosteroid is practically all in the etiocholane series In other words 11 hydroxyetiocholanolone and 11

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*Personal communication

ketoetiocholanolone arise from both E and F But from adrenosterone 11β hydroxyandrosterone and 11 ketoandrosterone are obtained as well as the 11-oxygenated steroids in the etiocholane series

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White I seem to have missed something I am not quite clear as to how these data are in harmony with the information given by Dr Pincus

Pincus All of the substances in the etiocholane series appear to be inactive as androgens All of the substances in the androsterone series are active

White When you say active was Dr Munson s bioassay in chicks?

Thorn That is correct

White This is interesting because a compound like androstenedione 3 17 while active in bioassay in chicks is distinctly less active in the rat Again one has to define what one means by androgenicity

Pincus In terms of bioassay, Dr Munson s data would suggest that there may be substances that synergise with the trace amounts of andro gen present

White Androstenedione is without known androgenicity in man in terms of trying to establish it as an adrenal virilizing androgen I am trying to limit the possibilities

Bush This substance which is so similar to 17 hydroxyprogesterone occurs in the urine of normal untreated females in about six times the amount found in the urine of normal untreated males

White Dr Bush as far as adrenosterone versus 17 hydroxyproges terone could not one distinguish between these two?

Bush Yes but so far this has not been done

White Can you differentiate by a color reaction i e for 17 keto steroids?

Bush No not in these tests

Pincus You haven t tried the Zimmerman reaction?

Bush No We have had only very small amounts of 17 hydroxy progesterone and 17 hydroxyprogesterone gives an indistinguishable color on paper I find I think Dr U Savard finds a different color

Pincus Have you had any indication in these venous effluents of C_{19} compounds?

Bush Not surely no but in one of the washing procedures I think anything less polar than desoxycorticosterone would suffer quite con siderable loss I have usually done a Butenandt separation which of course would lose androstenedione

Pincus Nelson and Samuels originally thought that they had 11 hydroxyandrostenedione or something like that in the blood from the dog and we are just starting a project for the isolation of C_{19} com pounds from adrenal perfusate There is no doubt in our minds that there are some there The only question is as to their chemical nature We are still very much in the dark

Thorn To revert back to the studies in regard to liver function we have been interested for some time in the effect of disorders of liver function on steroid metabolism Our technique has been to administer approximately 500 mg per day of a steroid to a patient with acute

hepatic disease We have usually given 1 5 to 2 5 Gm total of steroid Later when the patient has recovered we repeat the same dose and study the difference in the type and quantity of urinary steroids excreted under these two circumstances These studies we hope will assist us in our interpretation of changes in urinary steroids when liver function is impaired

We have had one interesting experience I hesitate to report a single case except that it seems to be so well documented Ordinarily 50 mg of cortisone given by mouth will induce a significant eosinopenia In a particular patient with severe hepatitis 50 mg of cortisone by mouth or intravenously did not produce an eosinopenia whereas 50 mg of compound F did Following recovery from acute hepatitis this patient showed a marked eosinopenia when 50 mg of cortisone was adminis tered It would appear in this instance that cortisone was relatively ineffective in producing an eosinopenia when administered to this patient in the presence of acute liver disease whereas ACTH and compound F gave the expected eosinopenic response both during the phase of acute hepatitis and later in recovery

Pincus That is what we were talking about the last time that corti sone probably is transformed to F in order to be effective or to some other substance

Long Does that occur in a joint cavity too?

Pincus We have injected both cortisone and F into a joint and tried to recover the products Cortisone is not transformed to F in the joint

Long Does it affect the disease process of the joint?

Pincus It doesn t affect the disease process in the joint

Long But F does?

Pincus Yes

White Cortisone does work in the eye doesn t it?

Lukens Yes the apparent difference between the local effect of cortisone in the joint and in the eye has not been explained

Pincus I have the impression from what I have heard from people working in this field that if cortisone is given in a very large dose into the inflamed joint there may be an effect but the dosage of F that is necessary is certainly much smaller

Astwood Yes but it is not an all or none phenomenon

Pincus We have not been able to find any significant amount of F in the few inflamed joints from which we have withdrawn fluid

Long Isn t this a particularly important point to emphasize that possibly the effects of cortisone are dependent upon a preliminary trans formation into F or maybe some other compound?

Lukens In studies which are in progress in Dr Hollander s clinic the actual conversion and location of these compounds is being meas

ured It looks as if the compounds were located in the tissues in different ways I am sorry I can t give the details but the extent to which they are attached to or absorbed by the cells of the joint membrane differs for these two compounds

Long I think this very important when one thinks of all of the attempts that have been made to demonstrate an effect of cortisone on isolated liver slices and other tissues Maybe a conversion to compound F, or something else is required in order to get the metabolic effect

Selye On the basis of our experiments with the pneumodermal test hydrocortisone injected directly into ordinary skin (outside the pneumodermal region) does have a definite though extremely slight effect upon the surrounding cutaneous structures This action is enormously enhanced if the skin is detached by the pneumodermal technique Presumably, local conditioning of the hydrocortisone effect by the dystrophy of this type of skin is responsible for the cutaneous atrophy thus induced The same is true of cortisone under these conditions although its effects are less pronounced and less long lasting I think that these observations plus the local effects of cortisone treatment in ophthalmology and injection of hydrocortisone into joints with limited local effects definitely prove that both cortisone and hydrocortisone have topical actions as such without having to go through the liver or other parts of the body in order to be activated

Conn Baker and Ingle have shown that same local effect of cortisone

Selye Yes I mentioned their experiments on the application of alcoholic solutions to the skin in the course of my presentation

Pincus Your work is in the rat There may be a species difference

Thorn When cortisone and hydrocortisone are given by mouth in equivalent quantities the 17 ketosteroids and the biologically active androgens derived from the two compounds are quite different

Selye I wonder to just what extent increased fixation in the tissues is an important factor there The most striking thing in our experiments with the pneumodermal technique was that hydrocortisone stayed so long at the place where it was injected because of its slow absorption rate No other steroid that we have tried so far stays in the pneumodermal wall as long as hydrocortisone

Thorn That was the acetate?

Selye Yes we gave it as the acetate The cortisone was also given as an acetate in this experiment

Thorn The metabolic effect of a given amount of cortisone acetate injected is quite different from that of hydrocortisone acetate

Selye I should like to know how we can be sure that this is not due just to better fixation or slower absorption of the compound without assuming any qualitative difference?

Thorn We assume that it is due to those things because the free com pound is much more active when injected than the hydrocortisone ace tate

Pincus I should like to point out that in the assay that Dr Dougherty described we have been comparing E F B and a number of other com pounds and to our very great surprise although we should have known it from Dr Dougherty s original remarks E has about one one hun dredth the activity of F in that test whereas corticosterone B is if any thing only slightly less active than F

Thorn Which test is employed in this instance?

Pincus That is the anti inflammatory or antiphlogistic test in which Dougherty injects into the same site histamine plus the corticosteroid We have quantitated that test for assay and the possibility is again suggested that the 11 hydroxy function is very important for the par ticular effect

White From Dougherty s data if one takes compound E as 100 then compound F is about 7 000 i e 70 times as active as E but com pound B is 5 or 6 i e approximately one twentieth as active as E

Thorn If you were using compound F as your standard you would state that corticosterone and cortisone were very much alike wouldn t you?

White Compound B is less active than cortisone There is one other local effect that I think is of interest Dougherty finds that whereas compound F causes lymphocytolysis *in vitro* cortisone does not

Pincus What does compound B do?

White I do not know whether compound B has been tried for lymphocytolysis *in vitro* Compound B has limited effect *in vivo* in so far as lymphoid tissue changes are concerned

Pincus In answer to Dr White s question if there is any basis for the difference between 11 hydroxy and 11 keto as between E and F it is certainly much more specific than the general polarity of the com pound that is involved

Long It certainly is

Bush It must be a more specific effect than simply the difference in solubility

Astwood Dr Bush if compound F is more polar than compound E why is it more slowly absorbed into the aqueous body fluids?

Bush I do not know

Pincus Polarity in this particular system isn t necessarily proportion ate to water solubility The systems that are ordinarily used are organic solvents propylene glycol toluol or something of that sort

Pincus We have tried combinations in the Dougherty test There doesn t appear to be any sign of antagonism That doesn t mean how

ever to exclude the greater affinity It merely means that the affinity may be so great that it just can t be overcome with the doses of cortisone used

Bush Quite a strong indication that these differences in effect may not be simply related to polarity is the recent work by Dr Schellman, at Utah in which he has studied the association of steroids with serum albumin It has been known for a long time that testosterone is com paratively insoluble in water but extremely soluble in serum albumin solution What he has found is that this association with steroids is dependent on short range van der Waal s forces as much as on inter action with polar groups Thus any simple rules based on their water solubility or their apparent partition coefficients in chromatographic systems may be extremely untrustworthy

Loew Are very special conditions needed for the lymphocytolysis shown by Dougherty?

White Not particularly The steroid is added to buffy coat of blood a blood smear made within 30 seconds and the number of altered lymphocytes counted after staining of the smear

Loew Is this published?

White He spoke about it here at the Macy Conference (31) several years ago and has a paper in press We tried for a number of years to demonstrate it with adrenocortical extract quite unsatisfactorily But he recently has been able to do this with compound F

White I don t know to what extent this has been confirmed There has been controversy over whether this happens *in vitro* Dr Schrek and Dr Feldman have observed these changes Dr Robertson in Eng land was unable to demonstrate it Dr Ingle have you had any experi ence with this?

Ingle No

Pincus Dr Hechter several years ago tried several steroids but was unable to get a direct effect of any

Loew I think Hechter got it when he added a part of lymphoid glands to the serum

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ADRENALECTOMY IN MAN*

F D W LUKENS

George S Cox Medical Research Institute
University of Pennsylvania

ADRENALECTOMY IN MAN is done either for therapeutic or investigative reasons There are three principal conditions in which it has been tried first carcinoma of the prostate or breast for which it may be regarded as palliative treatment The relief of pain and the modest degree of retardation of tumor growth are significant in some patients although the operation is in no sense a cure for cancer The second important indication for adrenalectomy in man is Cushing s syndrome about which Drs Sprague and Conn have previously reported at these meetings Here I think that adrenalectomy should be regarded as curative certainly if the best results obtained in this condition can be confirmed and repeated Third adrenalectomy has been tried in a number of clinics for severe hypertension In this instance the procedure is distinctly investigative It is in investigations on this aspect of adrenalectomy that I have been associated with Drs Wolferth Zintel Hafkenschiel Hills and others (1 2) A series of 69 patients have been subjected to adrenal surgery up to September 1 1952 which is the date I have used for preparing these data

The indications for adrenalectomy are listed in Table III The patients are severe hypertensives as shown by the high diastolic pressure These are patients who have not responded to intensive medical care which now includes the use of hexamethonium apresoline and other drugs for hypertension They are usually patients who are not suitable for sympathectomy or ones who have failed to respond to sympathectomy We demand adequate renal function as shown by the blood urea nitrogen (BUN) and phenolsulfonphthalein (PSP) excretion We were not as rigorous about this in the early days of the study and in fact we deliberately performed adrenalectomies on two individuals with renal

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insufficiency in the hope that it might give these very ill patients some improvement But now we insist on adequate renal function

Thorn Would you wish to qualify that statement? Is that on un restricted protein intake or not?

Lukens That is on an unrestricted diet

The last item in Table III progressive vascular damage simply means *that the damage of angina congestive failure retinitis and so forth* is adverse to the prognosis of the patient

TABLE III

Indications for Adrenalectomy

1	Diastolic BP 120 mm Hg or more
2	Under 55 years old
3	No response to intensive medical care
4	BUN less than 20 mg per 100 ml
5	PSP excretion at least 15% in 15 mins
6	Progressive vascular damage

As listed in Table IV 69 patients have had extensive adrenal resec tion Adrenalectomy has sometimes been done alone but in the majority of patients it has been combined with various forms of sympathectomy usually of the Adson type I shall not discuss the role of sympathectomy although it will have to be evaluated in the final analysis For the pres ent it may be noted that these patients have all had adrenalectomy followed in most instances by adrenal insufficiency 58 of the patients requiring substitution therapy After adrenal surgery 11 of the 69 patients needed no substitution therapy This means that 58 patients have had enough adrenal tissue removed to determine the effect of

TABLE IV

Data to September 1 1952

	No	Per Cent
A Patients with extensive adrenal resection (subtotal or total resection with or without various forms of sympathectomy)	69	100
Total mortality to date	17	25
B Of the first 34 cases		
1 No surviving 15 to 29 months	22	
2 Died in first year	9 }	35
3 Died after first year	3 }	

adrenal insufficiency on the hypertension Of 22 patients living (September 1 1952), 9 need no replacement i e, 13 are available for the study of the more prolonged effects of adequate adrenalectomy

The replacement therapy of these patients has been essentially that described at these Conferences by Thorn Sprague Conn and others (3) for Addison s disease At the time of removal of the second adrenal the pre and post operative treatment currently consists of 50 mg of cortisone the morning of operation (a little less than we used to give) and 100 mg of cortisone a day for one or two days after operation The dose of cortisone is then tapered to 50, 37 and 25 mg of cortisone per day within seven to ten days if all has gone well If total adrenalectomy is done 1 to 2 mg of desoxycorticosterone are added beginning the day after operation Generally speaking in these patients we have tried to use as little desoxycorticosterone as possible because of experimental studies showing that DCA is a hypertensive agent

For the final maintenance therapy of these patients 25 mg a day of cortisone and 1 to 2 mg of desoxycorticosterone may be used There is a range of dosage For example as little as 4 mg of cortisone has been sufficient in some patients and as much as 50 mg has been used for short periods of maintenance in others In connection with immediate postoperative care there is another point These patients have required let us say 100 mg of cortisone a day but Drs Sprague Conn and Shorr (4) have used 200 to 300 mg a day in Cushing s syndrome in connection with adrenalectomy Thus it appears that the cortisone requirement of patients with different diseases may differ considerably

Thorn We can confirm this statement but wouldn t you prefer to qualify it and say that it is not necessarily so much a difference in diseases as the fact that the patient with Cushing s syndrome has been adjusted for years to a very high level of hormone

Lukens That is better worded Thank you

Sayers Have you carried out adrenalectomies on patients with Cushing s syndrome and actually demonstrated this difference in requirements?

Lukens We have not done total adrenalectomy in patients with Cushing s syndrome in the last few years

Selye In addition to Cushing s syndrome can you mention any other disease in which the requirements are much greater?

Lukens I suspect that they would be somewhat greater in removing an adrenal tumor for virilism in spite of the lack of systemic disease I say that because in a few patients who have had tumors removed for adrenocortical virilism the severe postoperative febrile response that has occurred suggests the need for massive replacement therapy By and

large good replacement therapy with cortisone abolishes these severe and undesirable responses to adrenal surgery Would you agree with that Dr Thorn and Dr Conn?

Thorn I can only say that we have given large doses of hormone to these patients with good results

Conn I would say exactly the same thing as Dr Thorn I don t think anyone has done careful studies to indicate whether or not the Cushing patient requires a much larger amount than a hypertensive in the post operative period

Lukens You have given the larger amounts as a precaution and they have worked well?

Thorn We have observed a poor clinical response in some patients with as much as 100 mg of cortisone per day which from all of our experience with complete bilateral adrenalectomy in man in conditions other than Cushing s disease should be adequate It would therefore appear that the patients with Cushing s disease may require much higher levels of hormone following operation and that a reduction in dosage must be carried out much more carefully and much more slowly I have not had enough experience in regard to adrenal virilism independent of Cushing s syndrome to answer that point

Lukens There are one or two other things We have not noticed the undue sensitivity of these patients to the cerebral effects of cortisone which was described by Drs Sprague and Conn at the last Conference (3) The patients appear to tolerate 25 mg a day of cortisone with no unusual cerebral effect That may be just a coincidence A few of the patients have complained of stiffness of the joints when their therapy was reduced It is not known whether these are pre arthritics in whom the condition is revealed or just what it means This stiffness is con trolled by Addisonian dose levels of cortisone

Pincus May I ask if you have given any replacement therapy with hydrocortisone?

Lukens Not in this series

Sayers Dr Lukens I am interested in details You reduce the dose of cortisone and keep the dose of desoxycorticosterone acetate fixed?

Lukens At times we stop all therapy in a patient for a test period If subtotal adrenalectomy has been done and if the blood pressure is still high as it frequently is substitution therapy is reduced to a mini mum In the course of this reduction these patients may develop early symptoms of adrenal insufficiency and it is in that connection that the stiffness has appeared Desoxycorticosterone may or may not have been given

Selye You did not try varying cortisone and desoxycorticosterone and following their effects upon the joint lesions?

Lukens No Stiffness has been a minor symptom We know of no objective findings which could be followed

All patients have had an excellent appetite on these small doses of cortisone so much so that obesity has become a problem in some of them This is obesity without edema as far as we know Since obesity is undesirable in patients with cardiovascular disease we have tried to prevent it

TABLE V

Classification of Response to Adrenalectomy

A	Fall of blood pressure to normal (± 10 mm Hg) lying and standing Top normal blood pressure = 140/90
B	Elevated blood pressure lying but postural fall to near normal
C	No change in blood pressure but improved symptoms or signs
D	No change in blood pressure and no improvement in symptoms and signs

The response to adrenal surgery has been classified in our clinic for our own purposes as Table V shows In group A there is a fall of blood pressure to normal levels whether the patient is recumbent or standing and there is marked improvement in most of their symptoms The B and C groups have been benefited less but these patients are significantly improved as far as their symptomatology and capacity to live and work are concerned In that connection I would note our emphasis on the value of a postural fall of blood pressure There are quite a few patients with a high resting blood pressure who are very much relieved and whose cardiac size has reverted to normal apparently because of this postural fall It seems to reduce their cardiac burden The last group frankly recognizes the fact that there are many of these patients in whom no benefit can be obtained

Gellhorn What does postural mean?

Lukens The blood pressure falls on standing

Gellhorn Are these patients also sympathectomized?

Lukens Sympathectomy promotes postural hypotension but it will occur in patients with adrenalectomy without sympathectomy

Ingle Is the postural hypotension affected by desoxycorticosterone? I recall Dr Huggins saying that an occasional adrenalectomized patient treated with cortisone alone would develop postural hypotension which could be corrected by desoxycorticosterone

Lukens I believe that is true although we have not studied it In our effort to use as little replacement therapy as possible these patients

have been treated by the use of elastic stockings and girdles which relieve the symptoms of hypotension We have used these rather than hormones

Conn It has been our experience that postural hypotension in this instance as well as in Addison s disease is evidence of inadequate replacement therapy All patients who have a postural fall of blood pressure and are taking small amounts of steroids get into trouble when exposed to stresses as mild as the common cold When replacement therapy is raised to the point of postural hypotension being abolished the patient does not get into trouble with mild stresses but this dose may be sufficient to restore hypertension

Lukens Our patients have been maintained for a life outside the hospital they withstand the ordinary respiratory infections and so forth but they still have a modest degree of postural hypotension We are probably keeping them at minimal essential maintenance which is about the goal

In group D Table V there is no change in blood pressure and no improvement in symptoms and signs and this must be placed with category A in one s thinking When these two groups are associated it places a problem clearly before us There is a good response in some patients and no response in others

In talking about these responses I have so far spoken as if all hypertensives were the same which of course is not true We have used the Smithwick grouping of the severity of hypertension in order that our results might be comparable to those of other investigators As Table VI shows we have not operated on any Smithwick group I patients

TABLE VI

Smithwick group	Response to adrenalectomy A	B	C	D	Total per group	Mortality (patients)
1	0	0	0	0	0	0
2	5	8	8	0	21	0
3	6	4	3	0	13	1
4	4	8	12	11	35	16

They are not ill enough and the prognosis of hypertension is very good in the early stages (5) The favorable effects of surgery in groups 2 and 3 are apparent In Smithwick group 4 although the best type of response the A response can be obtained there is a significant mortality In fact in this group it verges on 50 per cent It will be less than this in the patients currently operated

Long Would you tell us something about what the Smithwick groups 1 to 4 are

Lukens The Smithwick classification of hypertensive cardiovascular disease is an effort to make a broad evaluation of the severity of the disease in its principal anatomical sites The disease particularly involves the heart the kidneys the cerebral vessels and the retinal vessels The areas involved and the degree of the damage are tabulated in considerable detail and from the results a final classification is made Smithwick group 1 is the mildest group 4 the most severe stage of the disease Omitting the detailed definition of certain terms Table VII gives summaries of the evaluation of the clinical findings and Table VIII of the use of this data for the classification of the stage of hypertensive disease The Tables have been based on a recent communication from Dr R H Smithwick Using this accepted procedure of classification group 4 comprises the patients with the most severe arteriolar disease Even though the mortality is negligible in our patients in groups 2 and 3 it would be a great help if we could select those hypertensive patients who would have an A type of response This is a key problem and I hope that this gathering will give me some suggestions As Table IX shows an A type of response has occurred in five patients who were on

TABLE VII

Factors Used in Grading the Cardiovascular Status of Hypertensive Patients (Smithwick)

Factors	Numerical Value
Cerebrovascular accident with or without minor residual *Abnormal* EKG Enlarged heart Impending cardiac failure PSP less than 25% in 15 minutes or less than 60% in 120 minutes Age 50 or over Mild angina	1
Cerebrovascular accident with residual symptoms * Frank congestive failure moderate angina PSP less than 20% in 15 minutes Unsatisfactory response to sedation	2
PSP less than 15% in 15 minutes	3
Nitrogen retention	4

Cerebral deterioration or hemiparesis

TABLE VIII

Classification of Hypertensive Patients According to the Criteria of Smithwick

Group	Numerical Grade	Other Factors
1	Less than 4	Eyegrounds grade 0 to 1 No change in cerebral cardiac or renal areas
2	Less than 4	Eyegrounds grade 0 to 1 with changes in cerebral cardiac and/or renal areas Eyegrounds grade 2 3 or 4 with or without changes in cerebral cardiac or renal areas
3	4 or more	Resting diastolic level below 140 mm Changes are present in cerebral cardiac and/or renal areas but they do not include the following (a) Cerebrovascular accident with marked residual symptoms (b) Frank congestive failure (c) PSP below 15% in 15 minutes associated with a poor response to sedation
4	4 or more	Resting diastolic level below 140 mm combined with one or more of the following (a) Cerebrovascular accident with marked residual symptoms (b) Frank congestive failure (c) PSP below 15% in 15 minutes combined with a poor response to sedation or Resting diastolic level of 140 mm or more

TABLE IX

Results of Adrenalectomy after 15 29 Months

Type of Response	13 Patients on Replacement Therapy	9 Patients Not on Replacement Therapy
A	5	1
B	3	2
C	5	6

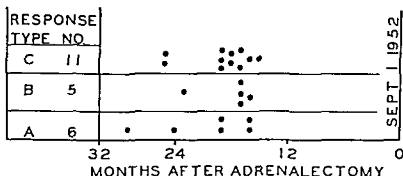


FIGURE 21 Twenty two patients followed for a year or more Type of response (A B C) is defined in Table V

replacement therapy and in one patient who did not need replacement therapy but who nevertheless had one of the best responses I can only say that she was an unusual patient Figure 21 shows the situation in graphic form This again focuses attention on the problem of the selection of these patients

Pitts In those that have been followed the longest with an A status initially has there been any tendency to shift to a B?

Lukens Many of those who have had an A type of response within three months—it may take some time to reach the optimum improvement—have maintained it as long as they have been observed On the other hand there have been patients who after an initially excellent response are not as well as they were

Of 7 patients with an A type response to adrenalectomy who lived more than one year four were in Smithwick group 4 One of them died at 15 months and three are still living indicating that even in the most severe hypertensives one can get this good result Two patients were in Smithwick group 3 one in Smithwick group 2

Figure 22 shows the course of S C the first patient in our series to have adrenalectomy for hypertension This shows the response in a patient with a class A reaction The high systolic and diastolic pressures in a control period and the normal blood urea nitrogen and the high normal urinary corticoids in this patient are recorded After subtotal adrenalectomy the systolic pressure fell fairly rapidly It took two months for the diastolic pressure to come down from 130 to 70 or 80 The time required for this response deserves attention The patient had a low corticoid excretion at the 6 and 12 month periods shown in the top part of the chart and then there was a high corticoid excretion at 24 months This was due to the fact that she became pregnant and was

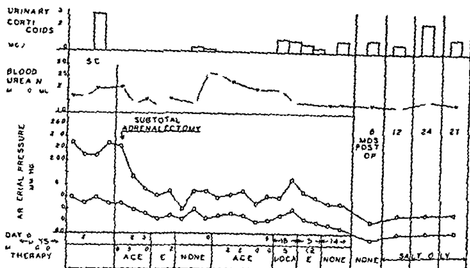


FIGURE 22 Patient S C Hypertension known for 13 months sympathectomy performed 9 months before subtotal adrenalectomy ACE = adrenal cortical extract (Upjohn) E = cortisone DCA = desoxycorticosterone acetate A portion of this Figure appeared in and is reproduced by permission from Lukens F D W and Wolferth C C Observations on subtotal adrenalectomy in hypertension *Tr Am Clin & Climatol A* 62 229 (1950)

capable of having the increased corticoid excretion of pregnancy It is interesting that this could occur in a woman who had a striking change in blood pressure She went through a normal pregnancy without any adrenal substitution therapy and without any change in her blood pressure or any other unusual event In not needing replacement therapy she is an exception to the general rule At 27 months, she reverted to her usual corticoid excretion Her blood pressure continues normal and she is fine

Atwood Was there a sympathectomy performed, too?

Lukens Yes she was one of the patients who had an extensive Smithwick s sympathectomy nine months prior to her subtotal adrenalectomy and the sympathectomy had failed to benefit her blood pressure as shown by the high initial readings (Figure 22) She is one of the patients who point up the fact that there is an adrenal cortical component in certain cases of hypertension just as Smithwick and others have demonstrated a sympathetic or neurogenic component of hypertension It is difficult at the present time to appraise the relative importance of these factors in the patient

Ralli How long had this patient s hypertension been present prior to the Smithwick operation?

Lukens Severe hypertension had been discovered four months before the sympathectomy and a year before adrenalectomy

Ralli Did her symptoms involve severe headache?

Lukens She had some headache Patients can tolerate so many types of hypertension that it is a difficult problem to decide on surgical measures There were no Smithwick group I patients in our operated series because those people have a long good prognosis Our effort is to select the very severely hypertensive patient before the stage of renal insufficiency

Long You just ventured the opinion that there was probably an adrenal cortical component here but during pregnancy when presumably the amount of let us say E and F present in the blood was greatly increased there seemed to be no effect upon the blood pressure

Lukens Yes

Long Is there any known adrenal cortical steroid which if given to this patient would bring the blood pressure back up? That it seems to me might be one of the best indications that one is dealing with an adrenal cortical component

Lukens That is answered in a rather inadequate way in Figure 22 Fairly soon after operation this patient had 5 mg of DCA daily for a period of 18 days That is a fairly large dose for an Addisonian although not massive It can be seen that there was a tendency for the blood pressure to rise We did not carry the experiment beyond that because we felt far more hesitant about doing things with these people then than we do now We have not investigated this to any extent for we have been trying to reduce substitution therapy to get the blood pressure down in the patients who did not respond rather than trying to bring it up in those who had a good response But we should do more such studies

Ralli Would increasing the salt intake in these individuals affect their hypertension?

Lukens We haven t forced salt We have given supplementary salt up to five grams a day for adrenal insufficiency to the patients in whom it doesn t raise blood pressure most of the time I think we would probably agree with Perera (6) that salt alone does not have a hypertensive effect when the originally elevated blood pressure has been reduced by adrenal insufficiency

Ingle Dr Lukens I recall seeing that patient within a few weeks after operation At that time she was still very weak Was she able to resume her usual household activities?

Lukens Yes she has resumed her full household activities including this pregnancy

Thorn To comment on Dr Ralli s question I believe that what salt

does in an adrenalectomized patient in relationship to hypertension may vary with the renal status If a patient because of renal disease is unable to retain salt within his body this may explain the apparent ineffectiveness of sodium chloride as a contributing factor to hypertension However it is not reasonable to exclude salt as a factor in the maintenance of hypertension in patients who retain appreciable quantities of salt Certainly from clinical experience one would deduce that the level of hypertension may be increased in many individuals in conjunction with increased salt retention

Lukens In that connection the effects of adrenalectomy and a regimen which might permit salt equilibrium at a different level should be related to the study reported by Dole (7) in which five patients had a very marked fall in blood pressure on a prolonged low salt intake We are studying that now hoping that some such test may differentiate the patients who will have the best type of response We have nothing to report at present

Dr Selye referred to Green s paper (8) on post DCA hypertension In that study Green tried adrenalectomy in hypertensive rats and it did not benefit the hypertension in these animals But in the same journal Fitts (9) reported that adrenalectomy abolished the hypertension of animals made so by a cellophane capsule about the kidney It is interesting to see in one copy of the journal this different response to adrenalectomy in two different types of experimental hypertension and to recall the fact that the patient likewise may or may not respond to adrenalectomy

Ralli Does the ketosteroid excretion in patients with severe liver disease depend on the compound that is given?

Thorn No it is decreased with almost any compound we have tested Would you support that Dr Conn?

Conn Yes

Ralli I mention this because the question arises whether some adrenal cortical factor is responsible for or contributes to hypertension or whether salt retention does so Patients with cirrhosis of the liver retain salt and are unable to excrete it normally Yet one practically never sees a patient with severe cirrhosis with an elevated blood pressure As a rule the blood pressure is low We have seen patients who were previously hypertensive and who following the development of cirrhosis of the liver have become hypotensive yet at the same time these individuals retain salt Apparently the retention of salt per se is not the cause of the hypertension or at least does not cause it when the secretions of the adrenal hormones are not increased

Lukens We certainly don t understand the detailed relation of salt or salt retention to this process

These experiences also raise the question of permissive action of the hormones because the best deduction I can make is that patients can maintain their blood pressure not only on a maintenance dose which could be permissive but on the total withdrawal of hormone up to the point of adrenal insufficiency The blood pressure may even be elevated to the point of actual circulatory collapse in some patients

Selye If I might add here also referring to your remark concerning Dr Green s paper I think one has to differentiate very clearly the meta hormonal type of post DCA hypertension and that which may be caused by the continued action of mineralo-corticoids We ran across this problem first in our work on the endocrine kidney There adrenalectomy tended to abolish the renal hypertension but even maintenance doses of adrenocortical extract sufficed to re establish it Hence we concluded that in this case the hypertension was actually due to some renal pressor substance and that the corticoids merelv exerted a conditioning effect in that they maintained the animals in a sufficiently good condition to allow the hypertension to manifest itself

In animals which developed high blood pressure as a result of short DCA treatment interruption of that therapy reduced the blood pressure to normal and they did not enter into a state of corticoid insufficiency Here the mineralo corticoid was apparently the primary cause for the maintenance of the high blood pressure level

In the metacorticoid hypertension which is what Green was working on the rise in blood pressure persisted long after prolonged DCA admin istration had been stopped In that instance the pressor effect apparently originated outside the adrenal (perhaps in the kidney) and the adrenal was not necessary for its maintenance as long as severe hypo corticoidism did not cause too much systemic damage

Lukens That raises a very important point as to the time in the course of the disease when adrenalectomv is worth while Our experience is that the duration of the disease makes no difference We have had good responses in people who have had the disease 10 or 12 years This should be post DCA if that is a factor

Selye It would be very difficult to be certain when the metacorticoid type of hypertension begins to develop and the adrenals are no longer of primary importance This is probably a matter of dosage conditioning factors etc What I meant to point out particularly was that although both types of experimental hypertension were primarily caused by an excess of DCA their mechanism and continued dependence upon corticoids is entirely different

Lukens Quite different We don t know what hypertension is in man

Pincus Didn t Dr Green report that hypophysectomy was effective in these animals?

Lukens Yes

Pincus You have not done a hypophysectomy?

Lukens We have discussed it but haven t done it I had heard that Dr Olivecrōna of Sweden had done hypophysectomy in hypertensive patients and lost them all

Ralli Our present resident in neurology at Bellevue spent some time with Dr Olivecrōna in Sweden and assisted him in some of the hypo physectomies in human subjects He reported to me that their cases did very well The only cases they lost were the malignancies that would have died anyway

Selye I had an opportunity to look into the records of some of these cases operated by Olivecrona with my friend Dr Rolf Luft this summer in Stockholm As Dr Ralli says at least those operated for carcinoma or diabetes were doing very well

Thorn I have had correspondence concerning a patient in Buffalo who was hypophysectomized in an attempt to correct malignant hyper tension The patient died during the last postoperative period Real difficulties may be encountered in the surgical removal of the pituitary in the absence of pituitary enlargement It is not easy to carry out a complete hypophysectomy It is a question whether or not patients who are so seriously ill as to justify hypophysectomy can in most instances survive one

Ralli Apparently the Swedish neurosurgeon Dr Olivecrona is very skilled with this procedure and does it with considerable success

Selye And may I say that as far as I could make out during my brief stay in Stockholm the operation is even less dangerous than that of bilateral adrenalectomy if it is performed under technically perfect conditions

Thorn So much of the postoperative convalescence would depend upon the ability of the neurosurgeon and the clinical status of the patient upon whom the operation was performed

Ingle Dr Lukens do those patients who fail to have lower blood pressure following adrenalectomy have advanced arteriosclerosis?

Lukens One cannot point to that as the distinguishing factor because many of the patients who responded have equally advanced arterio sclerosis We can t explain it at all

Pincus Haven t you been conducting urinary steroid excretion studies with these patients?

Lukens Yes A number of studies of urinary steroid excretion have shown that the hypertensive patient does not have any gross abnormality in steroid excretion and pending the development of new methods we are now using the few patients with total adrenalectomy to investigate the metabolism of steroids We fail to find any abnormality with present methods

Conn While we are discussing the question of permissive action of corticoids on hypertension and the possible relationship of this phenom enon to the sodium ion we might consider the hypertensive who develops Addison s disease Such patients then become hypotensive with their Addison s disease Administration of desoxycorticosterone restores the hypertension We have had the interesting experience of having such a patient who without any desoxycorticosterone but with cortisone alone, in amounts far too small to produce significant sodium retention developed severe hypertension That hypertension was produced by 20 mg of cortisone a day and that patient s blood pressure can be regu lated at will by reducing the dose of cortisone to 15 to 10 or getting up to 25 mg a day orally Thus there are other problems involved besides sodium alone or the hypertensive effect of desoxycorticosterone alone

Lukens There is no doubt about that We have been aware of the fact that cortisone may have a hypertensive influence We haven t studied it as accurately as you

Pincus Did you use corticosterone in that patient?

Conn No

Lukens We have seen a number of patients who were given 25 mg of cortisone a day and maintained a normal blood pressure and a num ber who needed 25 mg of cortisone a day but maintained a hypertension Thus there is still a problem of differentiation

Astwood Since in some patients one can give complete replacement therapy with cortisone and have a good response as Dr Lukens points out the permissive action in Ingle s sense is not sufficient to explain the response Another possibility is that the adrenal may be making some other steroid which contributes to the hypertension Could that not be tested by giving cortisone to cause adrenocortical atrophy as Wilkins and others have done in cases of the adrenogenital syndrome before taking out the adrenals to see whether there is a response?

Lukens We have watched the work of Dr Wilkins (10) with great interest because as you know he included the suppression of hyper tension in a patient with virilism by giving cortisone But with the few hypertensives that we have tested a half dozen or so we have never been able to lower blood pressure by giving cortisone We had hoped that that would be a diagnostic procedure Perhaps we should try it more extensively

Long How much cortisone have you given?

Lukens Well in one case we gave 200 milligrams That may have been too much

Thorn Yes

Lukens In some we have given smaller doses But we have never

Lukens Yes

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It has been interesting to observe that before the renal impairment becomes too great a significant improvement in the clinical condition can be brought about by bilateral adrenalectomy These patients rapidly lose their edema and are able to enjoy an unrestricted diet In one patient in this category who required sodium restriction before operation and who following operation was able to eat an unrestricted diet we tested the effect of desoxycorticosterone and cortisone On 37 5 mg of cortisone this patient could not be kept in mineral balance without supplementary salt intake On the other hand desoxycorticosterone re produced her cardiac enlargement and hypertension as did corticos terone Desoxycorticosterone was effective in this regard in less than 1 mg per day and corticosterone in a dose of 50 to 100 mg daily

Lukens This was a nephrotic?

Thorn This patient had had a long standing nephrotic syndrome but had within the past year developed severe hypertension with headaches

There is another point regarding the susceptibility of the hyperten sive patient to hormone withdrawal These patients have never previ ously experienced a complete withdrawal of adrenal cortical hormone from their bodies and in contrast to the patients in whom Addison s disease has developed slowly are extremely sensitive to the complete removal of cortisone In such patients a crisis can be precipitated in forty eight hours if all hormone therapy is suddenly discontinued In regard to the patient I have been speaking of we observed that on a maintenance dose of 37 5 mg of cortisone per day 1 mg of desoxy corticosterone every other day produced cardiac enlargement pulmonary congestion and the hypertension which was present prior to operation By discontinuing the 1 mg of desoxycorticosterone and adding 50 mg or 100 mg of corticosterone to the basic maintenance dose of 37 5 mg of cortisone we were able to reproduce the same changes as those with desoxycorticosterone I have no doubt but that Dr Conn is right and that perhaps in rare instances even a dose of cortisone as small as 25 mg may be sufficient to tip off this mechanism in some patients In the majority of patients however the Addisonian maintenance dose of cortisone 25 mg per day does not appear to be effective in restoring the changes that were modified by adrenalectomy On the other hand if one attempts to suppress endogenous adrenal hormone secretion in these individuals with 100 to 200 mg of cortisone per day the inhibition of secretion of desoxycorticosterone like factors might be more than offset by the hypertensive effect of the larger doses of cortisone

Bush The dosage of 37 5 mg of cortisone and either 6 grams of salt or 1 mg of DCA didn t restore hypertension?

Thorn No That is 1 mg of desoxycorticosterone acetate every other day in addition to the cortisone but without supplementary salt the

seen a patient with essential hypertension respond the way Wilkins patient with virilism did

Thorn Dr Perera reported on his observations some years ago He used, I believe, 100 mg of cortisone per day I should think that 50 mg per day particularly by injection would be more nearly the correct dose In commenting on Dr Lukens results it would appear to me that his group does much better therapeutically with the combined operations than when bilateral adrenalectomy alone is carried out In part this may be due to the more pronounced changes in blood pressure particularly in the upright position These changes occur of course with sympathectomy but are not often seen in our patients following bilateral adrenalectomy

Lukens *Our clinical impression agrees with that*

Thorn There is a very good possibility of that being an important element in your results The second point which I should like to discuss is that in some of the patients we have studied it is our impression that the important modifying influence of the adrenal in hypertensive vascular disease is almost completely lost when advanced renal complications are involved As renal disease progresses the modifying influence of the adrenal hormone appears to be less and less

A third point is that in hypertension and particularly in cases of chronic pyelonephritis when total daily sodium chloride intake is limited to five or six grams and the patient becomes depleted of body sodium and chloride reserves we have taken this as a contraindication for adrenalectomy because we feel that one of the specific benefits *which patients have derived from bilateral complete adrenalectomy* is that they can eat an unrestricted salt diet Thus if the hypertensive vascular disease is a type which does not require salt restriction we are less enthusiastic about undertaking adrenalectomy Ideal cases for complete adrenalectomy consist of those associated with marked hypertension and congestive failure or excessive salt retention where there is reasonable renal function Instead of constituting a contraindication I feel that cardiac failure in the presence of reasonable renal function is an indication for bilateral adrenalectomy Our trouble is much the same as that outlined by Dr Lukens in that radical procedures at this point at least must be reserved for patients with advanced disease On the other hand when the disease has advanced too far none of these procedures is particularly helpful

Another group in whom we have become interested is characterized by the middle aged patient with the nephrotic syndrome who having gone along for years with edema hypoproteinemia and normotension insidiously begins to demonstrate hypertension With the onset of hypertension in our experience these people run a rapid downhill course

and immediately following operation and that part of their immediate rehabilitation is obviously a cortisone nonspecific effect

Whereas the dose of cortisone used in most of these patients is higher than that used for Addison s disease maintenance therapy it has been the concensus of opinion that desoxycorticosterone is not necessary for many of these patients Thus it would appear that when because of cancer adrenalectomy is carried out a better balance is established by giving relatively larger doses of cortisone and smaller doses of desoxy corticosterone than is the case with patients with spontaneous adrenal insufficiency

Lukens Congestive failure may well be an additional indication for operation because the improvement in patients with congestive failure due to hypertensive disease has been remarkable Men who have had two or three bouts of congestive failure have been able to resume active work after adrenalectomy something that could not have been accom plished by digitalis in the majority of instances

In addition to the striking benefit in congestive failure there has been a significant improvement in hypertensive retinopathy This im provement comes late after a year at the earliest at eighteen months more commonly

We have not observed any significant changes in kidney function The few studies that have been done by Drs Clark Crosley and Barker (11) show that kidney function was about the same that renal plasma flow was about the same As this occurred at a lower level of blood pressure it meant that renal resistance had changed Similarly it has been determined that blood flow is the same in the brain at the lower pressure (12) Supplementary studies of this kind have been and are being made as we go along The matter of judging how much adrenal remnant there is and what response will occur is now actively in pro gress Dr Hills is using the various eosinophil response tests that you have outlined Dr Thorn

Long If subtotal adrenalectomies are done in the rat or the cat it is not very long before regeneration takes place and the same amount of cortical tissue as was present pre operatively is found In cases of subtotal adrenalectomy in man are we to suppose that the rate of regeneration in the human adrenal is markedly different?

Lukens I suspect that the regeneration differs in different patients Two or three of our patients were re-operated to have subsequent sympathectomy because their blood pressures did not fall At that time an adrenal remnant was removed and in all instances where that last remnant was removed one could see the difference in their need for therapy whether or not their blood pressure responded

Sayers How large was the remnant?

equivalent of 0 3 mg of desoxycorticosterone per day A dose of 0 3 mg of desoxycorticosterone every second or third day was as much of this hormone as the patient could tolerate without developing undue signs of salt and water retention

Conn Did you attempt to find a ceiling for cortisone alone?

Thorn No we haven t done that I would expect that a ceiling for cortisone would be soon reached if the dose went much beyond 50 to to 100 mg daily

It might perhaps be asked how we prove complete adrenalectomy Since most of these patients are on cortisone replacement therapy for long periods of time intensive ACTH stimulation must be given before one can assume that there is no residual adrenal cortical tissue In general we have relied on 3 successive 8 hour intravenous infusions of 25 units of ACTH each given on successive days We study the response not only of the eosinophils but of the 17 ketosteroids and 17 hydroxy steroids in the urine following this intensive period of ACTH stimulation We are not satisfied with the interpretation of complete adrenalectomy unless our values show no adrenal activation under these circumstances

There is one final point I should like to mention in regard to the discussion of bilateral adrenalectomy in the case of patients with prostatic cancer and metastases For some of the group who are not familiar with the clinical aspects of this procedure the theory behind adrenalectomy after castration was that there might be androgens secreted by the adrenals which would be favorable for the prostatic growth It was felt that by removing the adrenals completely it would be possible to eliminate this source of adrenal androgen In general I think the results have tended to confirm this in a high proportion of patients who thus far have been subjected to bilateral adrenalectomy There is the point however that cortisone replacement therapy does provide at least some biologically active androgen in the urine although the quantity involved may be smaller than that initially present prior to adrenalectomy For a matter of record I should like to state that very few biological androgen studies have been made on the urinary excretion of patients with prostatic cancer before bilateral adrenalectomy and following bilateral adrenalectomy on cortisone therapy However there appears to be no doubt but that the maintenance dose of cortisone used by most observers 50 mg per day following bilateral adrenalectomy has an over all beneficial effect on the patient

The second point to be considered of course is that although the interpretation of improvement might be on the basis of reduced circulating androgen in these patients following adrenalectomy it must also be realized that these patients received large doses of cortisone during

arterial disease? In diabetics the degenerative lesion is one of the most depressing aspects of the disease One almost feels as if the carbohydrate disturbance is in the nature of a red herring drawing attention away from the fact that in essence the disease involves severe degenerative lesions of the small arteries Patients with diabetes would seem to me to constitute an ideal group in whom to do complete adrenalectomy There is also the question of whether the retinopathy and arterial lesion in the diabetic is associated with an overproduction of adrenal cortical hormones or a sensitivity of the tissues to these hormones At times one feels that diabetes is perhaps primarily a disease of the adrenal cortex and that the pancreas is involved only secondarily!

Lukens It is commonly accepted now that diabetes has a metabolic component and some other component which we vaguely name degenerative As for doing adrenalectomy in such patients I have been wishing we could We have not encountered the appropriate patient yet

Long They should not be hard to find

Ingle Dr Green s patient who was adrenalectomized had severe diabetes and hypertension Vision was seriously impaired The eye grounds improved and the diabetes and hypertension were ameliorated following removal of the second adrenal

Lukens Yes that one improved and I can cite another Last summer Dr Paulsen of Denmark reported a patient with diabetes and advanced retinopathy who became pregnant and developed Simmond s disease and the diabetic retinopathy completely disappeared

Long Along with the diabetes?

Lukens The diabetes was modified but it did not completely disappear

Thorn While on paper this would appear to be a desirable group to work with we have all had some experience in the management of severe diabetes in an adrenalectomized patient and when complicated by hypertension this is not so simple

Lukens No it is exceedingly difficult and the only reassuring thing there is that most of the middle aged diabetics with hypertension probably do not have severe diabetes Less would be learned about diabetes but a more successful result should ensue than if the diabetes were severe

Ralli Am I to infer that these patients are very sensitive to insulin?

Lukens We have not tested their response to insulin If they have adrenal insufficiency I presume they are sensitive to insulin

Thorn We have had several patients with Addison s disease and diabetes and the manipulation of cortisone and insulin is very satisfactory in these patients most of the time However there has not been the added factor of hypertensive vascular disease which adds a worri

Lukens That varied in different instances The last one removed was an ample adrenal remnant that obviously could support life

Sayers This remnant had no medullary tissue?

Lukens No

Sayers It was entirely cortical tissue?

Lukens Yes The small amounts of adrenal cortex left would be far too little to contain any medulla

Long Had there been appreciable growth between the two operations?

Lukens That could not be determined because of scar tissue However in one instance it certainly was larger I think the real answer to your question is that many patients with subtotal adrenalectomy who require substitution therapy have required it for as long as they have been followed There is no regeneration in the sense that they escape the necessity for replacement therapy

Ingle In the rat extensive partial adrenalectomy can prevent the remnant from regenerating to normal size although it does undergo considerable hyperplasia

Lukens Many people who have adrenal insufficiency after subtotal adrenalectomy have a margin of reserve If they stop taking replacement therapy instead of going into crisis in one week it may take three or four weeks There is a small factor of safety which is not to be disregarded

Long The next question is whether this apparent slowness of regeneration of the remnant is related to the fact that in almost all instances substitution therapy has been given

Lukens No I don t believe that is the explanation Dr Long because we have always tried to give minimal supportive therapy in an effort to keep the blood pressure down The only exception to that is the fact that in the immediate postoperative period we have had to give ample therapy to get the patients well and out of the hospital If that stops regeneration then we have done it at that time but not later

Conn One might have a partial answer to your question Dr Long in those patients who have had subtotals for Cushing s syndrome where substitution therapy has not been added and where they have been clinically cured

Lukens That is true Dr Conn There are two types of patients the Cushing syndrome that has been totally cured and those that have recurred as a result of regeneration One cannot say in a single patient how much the adrenal remnant will regenerate

Ralli Have you had the possibility of doing this operation in patients with diabetes mellitus particularly those with diabetic retinopathy and

adrenalectomy? Did the cause of their hypertension lie in the adrenal glands or was it extra adrenal? It seems possible that in these patients the cortical hormones played only a permissive role in supporting the hypertension

There is another point which comes to mind a remark which I heard Dr Corcoran make a few years ago He said in effect There is some thing about being debilitated from any cause which is incompatible with the manifestation of hypertension not an exact quotation but the gist of it I wonder whether the relationship of the adrenal cortex to hypertension is more specific than this?

Long Dr Ingle I have often thought of this question in relation to your work on skeletal muscle where the asthenia as a result of the deprivation of cortical hormone is so very marked Might it not be that in adrenal insufficiency there is a situation in the smooth muscle of the blood vessels that is comparable to the asthenia that you so readily observed in the skeletal muscles? If this is so there might be relax ation of all the smooth muscles that go to make up the arterial tree

Thorn Except that patients with Addison s disease are known to be quite sensitive to pressor substances

Ingle Recent studies by Levine and his associates at Michael Reese Hospital may have a relation to that It was found that when mesenteric vessels of the adrenalectomized rat become unresponsive to nor epine phrine the responsiveness can be restored by the topical application of cortisone This group feels that the muscular asthenia of the adrenally insufficient animal is based upon circulatory failure We had made this suggestion years ago without direct evidence for it

Dr Lukens can you make a discrimination between the effects of adrenalectomy in hypertensive patients and the effects of injecting pyrogen?

Lukens First of all the injection of pyrogen would have short term effects These patients live in a steady state for months Another dis tinction is that the level of daily life and social usefulness of most of these people could not be supported if they were in a debilitated con dition So that while a general debilitated condition must be considered in one s thinking the level of that debility is rather mild

Sayers Dr Lukens you said something about the ability of these bilaterally adrenalectomized patients to maintain their blood pressure after withdrawal of replacement therapy Would you go into a little more detail on that? Am I correct in saying that when replacement therapy is withdrawn the hypertensive bilaterally adrenalectomized patient will maintain a higher blood pressure than a patient with Addi son s disease whose replacement therapy has been withdrawn?

Lukens Yes If a patient with a very high blood pressure has had the

some factor regarding hypoglycemic reactions It would appear in the group of patients we have studied thus far that adrenalectomy cannot be claimed to cause regression in the vascular changes, with the exception of changes in the fundi which appear in many instances to be *ameliorated if there is reduction in the blood pressure* by any means One of our best results was obtained in a young man who had been disabled for several years because of his hypertension and cardiac changes He returned to full activity for over a year but died suddenly approximately 15 months after total adrenalectomy Autopsy showed widespread coronary artery disease There seemed to be no evidence in this individual of any regression of his vascular changes

Lukens Most of our patients who have had angina pectoris are surprisingly little relieved Those who have had congestive failure are *much relieved* And that distinction may be of some importance to our *thinking*

Selye I should like Dr Ingle to comment on the role of the adrenal in this kind of hypertension if he will In particular for instance if both adrenals are removed and the patient maintained in good condition on the necessary corticoid therapy and it is seen that, despite the adequate corticoid treatment the hypertension does not return how do you interpret this? Would you feel that fits in with your concept of the purely permissive action of corticoids in this disease? Furthermore when a subtotal adrenalectomy leaves only a small remnant just sufficient to maintain the patient in apparently good health but insufficient to maintain a pathologically high blood pressure level how does that fit in with your concept of a merely permissive action? Or wouldn t you apply your theory to this kind of case?

Ingle I was more willing to apply it before this Conference began than I am at *this point*

Lukens I think *in some instances there is no permissive action* and *in others there might be permissive action at very different* levels *in* different patients which raises a special question for the investigator

Ingle *I have thought that the cortical hormones play a permissive* role in the maintenance of experimental renal hypertension Renal hypertension is ameliorated by adrenalectomy and yet the exciting cause of the hypertension is extra adrenal The fact that it is not fully manifest in the adrenally insufficient animal seems to me to be an example of permissive action

Drs Lukens and Thorn have found that in some patients the production of adrenal cortical insufficiency fails to lower blood pressure In such cases it would not seem that the adrenal cortices have played either an etiologic or a permissive role in the hypertensive state But what should we say of those patients whose hypertension is ameliorated by

adrenalectomy? Did the cause of their hypertension lie in the adrenal glands or was it extra adrenal? It seems possible that in these patients the cortical hormones played only a permissive role in supporting the hypertension

There is another point which comes to mind a remark which I heard Dr Corcoran make a few years ago He said in effect There is some thing about being debilitated from any cause which is incompatible with the manifestation of hypertension not an exact quotation but the gist of it I wonder whether the relationship of the adrenal cortex to hypertension is more specific than this?

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Lukens Yes If a patient with a very high blood pressure has had the

adrenals removed and if that patient does not respond and the hypertension continues then even on withdrawal of all replacement therapy the hypertension continues up to the point of circulatory collapse when obviously it goes down

Conn But that is the time when adrenal steroidal activity ceases isn t it?

Lukens One can very well argue that point It is difficult to determine at what point all the cortical hormone disappears

Conn But when the patient has that vascular collapse and is given some cortical hormone the blood pressure comes up again

Lukens That is right

Conn So that as far as permissive action is concerned I think no conclusive evidence has been presented today against it

Lukens Not if you recognize the fact that that permissive action is taking place at different levels of adrenal hormone In other words of an imaginary 10 patients on the same maintenance therapy 5 have a normal blood pressure and 5 have hypertension so that the permissive effect of that particular dose is not uniform

Selye Would Dr Ingle agree to using his term in the sense of envisaging permissive actions occurring at various dose levels? If we are going to say that a certain action is permissive and yet can occur at various dose levels then all physiologic phenomena can be visualized as permissive I should have thought that the term could only be used for yes or no responses as originally suggested by Dr Ingle i e a factor either does or does not permit an effect to occur but never alters the degree of that effect In a dose response relationship then I think the term permissive in its original formulation can no longer be applied

Lukens Well not as rigidly as one does in the laboratory

Loewi Speaking only of the physiologic actions of adrenocortical hormones not of their pharmacologic actions what is meant by permissive action? In 1933 a very important paper was published from Dale s laboratory (13) The authors observed that starving rabbits died from hypoglycemia one or two days after hypophysectomy although the liver and muscles contained a lot of glycogen The authors concluded that the animals apparently could not mobilize glycogen To prove the correctness of this view they injected such animals with epinephrine it failed indeed to mobilize glycogen Later they found and this was confirmed by others that after hypophysectomy mobilization from the stores of fat is inhibited too This inhibition occurs because in hypophysectomized animals cortical hormones are no longer secreted Thus the mobilization of fat is inhibited also in adrenalectomized animals Finally it was shown that mobilization of protein is also inhibited under the same conditions If the mobilization of protein was not inhibited

in starving hypophysectomized or adrenalectomized animals the blood sugar would not drop It does not drop in starving normal animals as they are capable of mobilizing protein for the formation of glucose From all these results it became obvious that the adrenal cortex is needed for the mobilization of foodstuffs

Other physiologic actions of the cortex were demonstrated by Ingle He showed that after adrenalectomy stimuli which in normal animals produced special effects were no longer effective Among such stimuli were fracture of bones asphyxia and cold However these stimuli were effective in adrenalectomized animals if they had received physiologic doses of cortical hormones

I came to the conclusion that the physiologic action of cortical hormones consists of maintaining the normal responsiveness of organs to metabolic stimuli Why is this called a permissive action? The ability of insulin to maintain the normal blood sugar level is not called a permissive action It is a physiologic action I don t see any reason why one should call the action of the cortical hormones a permissive rather than a physiologic action

Long I raised this same question earlier because I have had certain doubts on the semantics myself What is the difference if there is a difference between a permissive and a physiologic effect? I can recognize the effect of no hormone at all and I can recognize the effects of an excess But under physiologic conditions presumably the amount of cortical hormone is placed somewhere between those two extremes

Thorn I too have some questions I believe that most of us are using 25 mg of cortisone per day in the hypertensive patients following complete adrenalectomy

Lukens That is a common dosage

Thorn Does 25 mg of cortisone in hypertensive patients represent more or less than these patients secreted prior to their adrenalectomy? This may be important because in grading how a patient feels we know that if the cortisone dosage is greater than his own adrenal was customarily putting out prior to operation part of his improved sense of well being may be the so called nonspecific effect of cortisone and might he not feel better on 25 mg of cortisone with his adrenals intact This is something we must think about and may need to evaluate subsequently

A second problem relates to studying these patients with smaller doses of hormone or with no hormone whatsoever We know that the sudden withdrawal of hormone can precipitate a crisis On the other hand we might reproduce the spontaneous development of Addison s disease by very slowly reducing the hormone over a prolonged period of time and in this way see if the individual s system would adjust to

small quantities of adrenal steroids If sufficient sodium chloride is provided I am certain it would be possible for these patients to get along on very low levels of hormone I doubt whether the patient would feel as well as he does under cortisone therapy, but it would provide an opportunity to explore Dr Ingle s concept of the permissive aspect of adrenal cortical hormone therapy

Ingle Dr Thorn are there any adrenalectomized, hypertensive patients whose blood pressure cannot be re established at the pre adrenalectomy level by the administration of cortisone alone?

Thorn I can t say because we haven t given them unlimited quantities of hormone Certainly 25 to 50 mg per day does not restore the preoperative signs and symptoms of many of our patients

Ingle Here is a very basic question Does adrenalectomy remove an agent which has caused the development and maintenance of hyper tension?

Lukens I don t know anything about the cause of hypertension

Ingle Would you venture a guess as to what the role of the adrenal cortex may be in the etiology of hypertension?

Lukens It is more likely to be supportive than causative

Long Yet a fair number of individuals with Cushing s syndrome have hypertension do they not?

Lukens The majority of them

Long So that an excess of something from a human adrenal will cause hypertension and removal of the tumors or the gland or perhaps the pituitary itself will relieve it

Lukens I think both Cushing s syndrome with its hypertension and the response of some patients with hypertension to adrenalectomy suggest that the adrenals may be an important factor in either the cause or the maintenance of the hypertension in a limited number of instances But limited number of instances must be emphasized because certainly there are many forms of human hypertension that seem to be independent of that mechanism or relatively independent

Ingle One might also say that the same is true of the relationship of the adrenal cortex to diabetes There are a few in which it is clearly a factor

Have you formed any opinion which you would care to express Dr Thorn as to the relationship of the adrenal cortex to the etiology of hypertension?

Thorn I would be willing to say this in the majority of people whom we see with advanced hyptertensive vascular disease the most important single factor is probably not the adrenal cortical hormones second the adrenal cortical hormone is certainly a factor in the main tenance of hypertension and particularly is it important before the onset

of high grade renal insufficiency However once renal insufficiency is well established there would appear to be less relationship between the absence of adrenal cortical hormones and the maintenance of hyper tension

Sayers In Cushing s syndrome isn t it true that in a large percentage of these patients there is some renal damage? What relationship does that renal damage have to the hypertension that develops? I read a review by Eisenhardt and Thompson (14) in which a large series of these patients came to autopsy and if I remember correctly about 50 to 70 per cent had a fair degree of renal damage I am interested in what comes first the renal damage or the hypertension

Thorn Throughout the United States in the past year or two there must have been a large number of patients in whom Cushing s disease was induced with excessive cortisone therapy As far as I know, the incidence of hypertension is relatively small It is my impression that the percentage of hypertension in patients with spontaneous Cushing s disease is much greater although this would certainly need statistical analysis Whether or not such statistics would be helpful in elucidating the point that the total adrenal secretions are more conducive to hyper tension than cortisone alone remains to be seen

Long Have you produced Cushing s disease in the strict sense of the term with cortisone? My impression from reading the literature is that in a large group of people treated with cortisone some develop hypertension some the moon face and some hirsutism but that nobody so far has gone to the extreme of continuing the cortisone once the first sign of excess dosage appeared so it is quite different from the natural disease

Thorn We have continued cortisone in the face of signs of excess dosage when very severe underlying disease has been present and there seemed to be no other means of helping the patient These patients have then been maintained with obvious Cushing s syndrome for fairly pro longed periods of time

Long That would be the group to compare with the natural disease

Pincus I believe that Dr Sprague in his comparison of the effects of cortisone in Cushing s disease found practically every symptom with I think two minor exceptions

Long But not all in the same individual

Pincus That is true

Thorn Not all the patients with Cushing s syndrome have all of the manifestations of the syndrome

Conn No that is right About 30 per cent of them don t have diabetes

Thorn And some patients do not have hypertension

Lukens To return to your question Dr Sayers, there is no doubt that there is a renal component in the hypertension of Cushing's syndrome at least in its later stages but in citing autopsy statistics one presents the worst possible picture as far as the kidney goes Many of these patients at an earlier stage had clinically normal renal function

Ingle What is the incidence of hypertension and of diabetes among patients with gigantism and acromegaly? I am thinking of the clinical implications of Dr Selye's recent studies with growth hormone

Ralli Cushing and Davidoff (15) reported an incidence of diabetes of about 75 per cent in patients with acromegaly We have observed three patients with acromegaly all of whom had diabetes interestingly enough never complicated by ketosis The patients did have elevated blood sugars and elevated blood cholesterols The one that is still living a male does not have hypertension

Lukens Figures in the literature range from 12 per cent to the high frequency you mentioned

Thorn Do the stigmata of acromegaly persist after the active phase of acromegaly has disappeared?

Lukens That is true

Thorn It is a little difficult to evaluate the absence of diabetes under these circumstances

Lukens I think it is generally agreed that acromegaly does not produce an increased incidence of hypertension

Long So far as diabetes in acromegaly is concerned the statement has been made—it may have been made by Dr Cushing—that since the course of the disease is long, the patient is frequently seen in the so called burned out stage Probably all acromegalics have glycosuria at some time during the disease

Lukens That could well be

Ralli We had one patient who had acromegaly and did develop hypertensive retinopathy This patient died of a cerebral hemorrhage and at post mortem a pituitary tumor was found which showed both eosinophilic and basophilic cells There has been a good deal of discussion about the amount of hormone that is needed by adrenalectomized patients but I have not heard whether when these people return to ordinary everyday life the need for hormone goes up Suppose the man goes back to his work of bricklaying or whatever it is and he has been stabilized on 25 mg of cortisone a day is there any indication that he must be given more cortisone?

Lukens It is difficult to answer that because we are constantly adjusting our treatment to these patients needs I might mention that the first hot summer our patients went through we were watching them all the time and we had one or two minor accidents but the last

two summers we had no extra visits to the clinic by any patient

Long And you have not had to raise the hormone?

Lukens We have not had to raise the hormone appreciably That goes for intercurrent respiratory infections or their usual occupations I am sure however that if we had the hormone especially adjusted at some very low level we might have to raise it It may be that we are not measuring the thing accurately enough to bring out the answer to your question

Long Dr Thorn raised this point a few moments ago when he was asking the question as to whether 25 mg was too much or too little The experience seems to be that roughly 25 to 37 5 mg a day which were the figures you were quoting is ample in this series of patients although of course I have no information as to what their occupations were But you say they go through intercurrent infections without the necessity of the hormone being increased?

Lukens Yes

Long Certainly the Addisonian on 25 mg of cortisone would require more if he had an intercurrent infection

Conn That is true in our experience We have had very frequent experiences in which patients who had been maintained on 20 to 25 mg and 1 mg of DCA have had to be increased to 35 or 50 mg of cortisone usually not above 50 in the face of intercurrent infection

Lukens We have had one or two crises precipitated by more severe intercurrent infections It is hard to define intercurrent infection

Thorn The problem partially revolves around the inability of the Addisonian patient or the adrenalectomized patient to compensate for physiologic or pathologic changes that are induced by a particular in fection For instance the presence of diarrheal disease results in the loss of a large amount of sodium chloride and water and under these circumstances it may be essential to give a very high level of adrenal substitution therapy whereas a patient could engage in heavy phy siologic activity per day such as walking or chopping wood without perhaps the necessity for such a marked increase in his hormone re quirement It would appear that the patient with Addison s disease or an adrenalectomized patient can tolerate physiologic increases in work with only a small increment in hormone whereas pathologic changes such as dehydration may require much greater adjustment

Long You say that a man balanced on 25 mg a day in the hospital can go out and chop wood That means he can raise his basal oxygen consumption from 250 ml a minute up to say 3 liters a minute a very large increase in the metabolic rate Nevertheless so far as you know there is no indication that there is anything like a proportional increase in the requirement of cortical hormone The question of utilization has

come up here time and time again and it is important in understanding the action of the hormone It seems to me that the adrenalectomized human affords us an excellent opportunity to find out exactly how much greater is the need for hormone within the physiologic range Exercise may be one of the best ways to test it

Selye Another aspect of the same problem is the role of the adrenal cortex in adaptation Our first animal experiments which served as the basis of the adaptation syndrome concept relate to this point one which is also important if we consider what Dr Loewi said a few minutes ago about the role of the adrenals in maintaining a physiologic responsiveness of the tissues In those experiments we showed that if a rat is previously adapted to perform violent muscular exercise to live in the cold or to be treated daily with a certain drug it will retain much of its increased resistance even following subsequent adrenalectomy Animals adrenalectomized prior to acquiring adaptation to such stressors have of course a very low resistance to them It was somewhat unexpected that continued hyperfunction of the adrenal cortex was not necessary for instance to transform protein into sugar as a fuel for intense muscular work once adaptation had been acquired or to maintain the body temperature through metabolic processes despite exposure to cold However that is what we found Adrenalectomized animals even if maintained only on sodium chloride did preserve a great deal of their acquired resistance to all these stressors after adrenalectomy

It is hardly possible to select any one biologic phenomenon as being the only one for which the corticoids are necessary However it does seem to me that the above experiments show that the adrenal is not so much required for maintaining a responsiveness of the organs (after adaptation the organs appear to remain responsive to certain stimuli even in the absence of the adrenals) but for the acquisition of adaptation

Long That brings us back to a point discussed before What you say about the adrenal may be perfectly true but I think the phenomenon involves a great deal more than the adjustment to the adrenocortical secretion

Selye Oh no doubt What I meant to show by these experiments was that during the initial stage of the G A S (the alarm reaction) when the organism first meets with circumstances to which it has not been adapted it needs a great excess of corticoids Later during the stage of resistance it needs much less I would think that this is so because the peripheral cells—in the case of adaptation to muscular exercise the muscle cell itself—can go on doing work quite efficiently with a minimum of corticoids if the organism had produced large enough

amounts during the initial stage while adaptation was being acquired

Long Couldn t you make out almost the same case for the thyroid hormone? That in adaptation to cold large amounts of thyroid hormone are required

Selye Has it ever been shown that an individual previously adapted to cold requires less thyroid hormone to resist cold subsequently and can do so even after thyroidectomy?

Long I don t know

Lukens In connection with adaptation these patients are hospitalized for a fairly prolonged period so that adaptation to life outside the hospital is slowly achieved They are not subjected to a sudden test in that sense

Sayers Do you return these patients to an active life as soon as they get out of the hospital? For instance would a chap who has been active as a bricklayer go back to his occupation as soon as he is out of the hospital?

Lukens No and the chief reason is the orthostatic hypotension that most of them have It takes two or three months for them to adapt to that

Thorn Our patients with complete bilateral adrenalectomy alone do not experience orthostatic hypotension

Sayers And you have seen a number of bilaterally adrenalectomized individuals do a considerable amount of muscular work without getting into difficulty?

Lukens Yes I would say so

Thorn We had one patient who had been disabled for over two years with hypertensive cardiovascular disease who within a month or two after his bilateral adrenalectomy was carrying on hard physical work

Selye Was this a person previously accustomed to muscular work of the same kind?

Thorn This patient was a well developed young man but he had been a complete invalid for two years He had been very active earlier in his life

I would like to ask the group to interpret a phenomenon that we have observed of a marked fall in eosinophils following complete bilateral adrenalectomy in a patient who was given a constant large dose of adrenal hormone throughout and following the operation With a constant intravenous infusion of compound F there was an eosinopenia followed by an eosinophilia The interesting aspect of this experiment is that with continuing constant infusion of hormone in a patient without adrenals there was an eosinophilic rise to the pre-operative level

Long That is the question I raised previously if an adrenalectomized animal was given a constant amount of hormone and exposed to stress would the eosinophils fall?

Thorn The quantity of hormone given during the operation in this instance could explain the fall in eosinophils However the magnitude of fall was similar to that seen spontaneously in patients with intact adrenals subjected to major surgery

Long They follow the same curve on a constant amount of F?

Thorn After the first day of operation the patient was kept on a constant quantity of hormone The eosinophil response seemed to break away from the constant infusion of hormone at a time when one would expect the hormone to be in excess of the patient s needs We ourselves have been in error in interpreting this rebound in the patient subjected to surgery with intact adrenals as necessarily reflecting a state of adrenal inactivity or relative insufficiency of hormone I would doubt that on the seventh day or eighth day with 100 mg of F having been injected intravenously around the clock that one could assume adrenal cortical insufficiency was present at that time

White This individual had what clinical state?

Thorn The patient I am reporting on was a hypertensive individual subjected to bilateral complete adrenalectomy

White Is there evidence that on the seventh day the individual was still under what could be termed stress? Also why are we surprised by this rebound phenomenon?

Thorn We were surprised to see the rebound eosinophilia with the high level of hormone administered at this time

Selye The statement has been made by several investigators that after adrenalectomy stress often tends to cause an eosinophilia Preliminary experiments performed at our institute suggest that STH may counteract the eosinophilia normally produced by cortisone One wonders whether endogenous secretion of STH may be involved in such responses Has anybody here evidence on this type of action either in animals or in man? This would seem to be one possible compensatory mechanism designed to maintain a normal blood eosinophil level

White The situation is one in which adrenal corticoid steroids are undoubtedly being used for purposes other than the production of an eosinopenia and the requirements of the tissues for adrenocortico steroids for these processes may influence how much of the adreno corticosteroids are available for affecting eosinophils That is why I asked whether the individual at the seventh day was still under a stress If so his tissue utilization of hormones might be larger than supposed The situation might be compared to the fact that in every circumstance in which a lymphocytopenia results there is set into motion various factors about which we know very little to restore the lymphocytes to normal As a result of this production of new cells which are being made to replace cells that have disappeared there is invariably a re

bound phenomenon perhaps a reflection of a sudden rush of production

I think there are three factors first what the hormone does to the cells being counted which are disappearing under the hormonal influence second how much hormone there is for this specific function and third the rapidity with which the new cells being counted are being replaced i e the production factor This is seen to a particularly striking degree following adrenalectomy Perhaps one of the best examples is experimental radiation If a normal mouse is exposed to 200 R under specified conditions there is a striking lymphocytopenia and involution of lymphoid tissue 48 hours later the lymphocytes have not come back and the lymphoid tissue has only begun to return to normal size and structure But with the same dose of radiation in an adrenalectomized animal 24 hours later the lymphoid tissue has not only come back to normal size and structure but there is actually a lymphoid tissue hypertrophy Thus the observations we have been talking about are perhaps not surprising and a possible explanation it seems to me is that eosinophils in the blood represent only one of the variables

Thorn The interesting aspect of this experiment and one of the points I wanted to illustrate was that at the time the eosinophils were rising and one might say there was an escape from adrenal hormone effect there was a virtual absence of sodium in the urine Thus the 100 mg of compound F given around the clock did not permit the usual escape of sodium that might have been anticipated at this point

Long There are studies in the literature which show that very soon after the eosinophils fall in the blood there is marked stimulation of the eosinophilic elements in the bone marrow presumably the longer the eosinophils are lowered in the blood the greater the stimulation for the new production of eosinophils will be I believe Spears and the group at Wisconsin have published simultaneous bone marrow blood eosinophil counts To follow through on Dr White s suggestion it may well be that in the period of eosinophilia the bone marrow was intensely active in the production of eosinophilic cells

Sayers Another example where one gets into difficulties with indices when studying changes over a considerable period of time is changes in ascorbic acid concentration in the adrenal Initially following the application of a stressful stimulus a marked depletion of ascorbic acid occurs in the gland If the stimulus is applied continuously the ascorbic acid concentration will in many instances be restored to normal and even reach higher than normal concentrations Dr Long s group has shown that and we have had it appear in many instances in our own experiments I think what happens is that the gland synthesizes ascorbic acid at a more rapid rate

Long I think that is correct because in our experience and I believe in yours at such a time the cholesterol content of the gland will be much depressed and the gland will be enlarging although the ascorbic acid would lead one to believe that a normal gland is being dealt with

Pincus Do you follow the uric acid in these experiments?

Thorn We do follow the uric acid excretion but I don t have the values in this instance We propose to bring this patient back and repeat the intravenous infusion of the same quantity of F without the operation in order to detect whether utilization or alteration in the metabolism of the hormone takes place

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